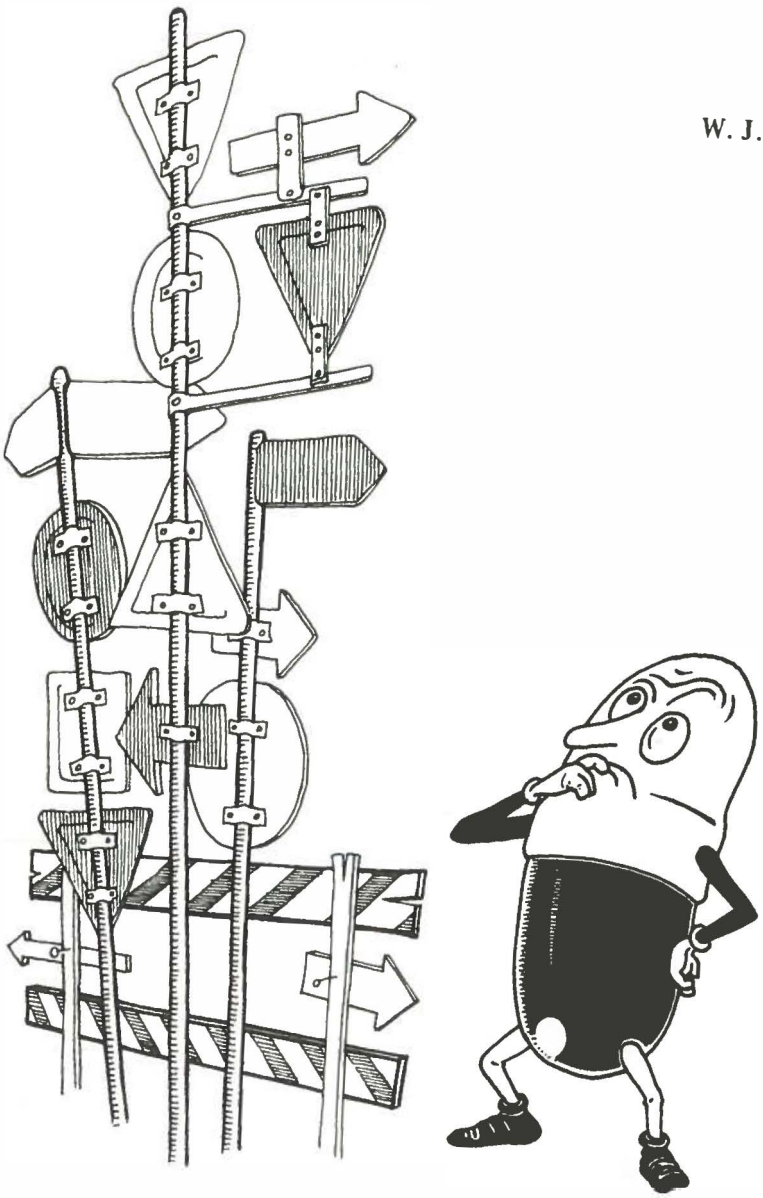
Pharmacological studies of dantrolene sodium, a muscle relaxant for the treatment of spasticity

Meijler, Willem Jan

PHARMACOLOGICAL STUDIES OF DANTROLENE SODIUM, A MUSCLE RELAXANT FOR THE TREATMENT OF SPASTICITY.

W. J. MEIJLER



PHARMACOLOGICAL STUDIES OF DANTROLENE SODIUM, A MUSCLE RELAXANT FOR THE TREATMENT OF SPASTICITY.

Tekening omslag: Bert Cornelius.

STELLINGEN

behorende bij het proefschrift van W.J. Meijler

1. De behandeling van spasticiteit beoogt een verbetering van de functionaliteit, hetgeen niet gepaard hoeft te gaan met het opheffen van de functiestoornissen.

2. Alcoholgebruik vormt voor spastische patienten een extra probleem vergeleken met niet-spastische mensen.

3. In een open onderzoek, waarin dus zowel het intrinsieke als het placebo effect van het middel een rol spelen, heeft een negatief resultaat meer betekenis dan een positief.

4. In het kader van de integratie van gehandicapten in de samenleving verdient het aanbeveling om revalidatiecentra naar het centrum van woongemeenschappen te verplaatsen.

5. De overdracht van wetenschappelijke informatie kan worden verbeterd door gebruik te maken van de visuele hulpmiddelen zoals die ook in de reklame worden toegepast.

6. Voor de overconsumptie van geneesmiddelen is allereerst de arts verantwoordelijk, die door onwetenheid, onverschilligheid en zwakheid te veel voorschrijft; de patient, omdat deze onduidelijk is over zijn klachten; de farmaceutische industrie, die zinloze medicijnen produceert; en tenslotte de overheid, die weinig doet om deze situatie te veranderen.
J. Bernard, openingstoespraak IUPHAR-congres, Parijs 1978.

7. Het is gewenst dat in de postdoctorale fase van de medische opleiding, naast de "differentiaal diagnose", ruime aandacht wordt besteed aan de "differentiaal therapie".

8. Een medicijnenwinkel, die informatie verstrekt over geneesmiddelen, verstoort niet de arts-patient relatie, maar levert een bijdrage tot de mondigheid van patienten.

9. Bij de fusie van scholen wordt te veel rekening gehouden met de personeelsproblematiek ten koste van onderwijs inhoudelijke aspecten.

10. Op grond van een kostenberekening van het krantenbedrijf zou men tot de conclusie kunnen komen, dat journalisten zijn aangetrokken om de achterkant van reklameboodschappen vol te schrijven.

Groningen, 22 november 1978.

RIJKSUNIVERSITEIT TE GRONINGEN

PHARMACOLOGICAL STUDIES OF DANTROLENE SODIUM, A MUSCLE RELAXANT FOR THE TREATMENT OF SPASTICITY.

Proefschrift

TER VERKRIJGING VAN HET DOCTORAAT IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE GRONINGEN OP GEZAG VAN DE RECTOR MAGNIFICUS DR. J. BORGMAN IN HET OPENBAAR TE VERDEDIGEN OP WOENSDAG 22 NOVEMBER 1978 DES NAMIDDAGS TE 4.00 UUR

door

WILLEM JAN MEIJLER
geboren te Groningen

PROMOTOR: DR. H. WESSELING

REFERENT: DR. A.H.J. SCAF

COREFERENT: DR. J.P.W.F. LAKKE

Any intelligent fool can invent
further complications, but it
takes a touch of genius to
attain - or recapture - simplicity.

E.F. Schumacher.

CONTENTS

Samenvatting.

Spasticiteit kan bij patienten zó'n beperking veroorzaken van hun functioneren, dat het de moeite waard is te proberen de spasticiteit te verminderen. Hiervoor staan verschillende methoden ter beschikking, waarvan de farmacologische beïnvloeding één van de belangrijkste is.
In dit proefschrift wordt eerst een korte samenvatting gegeven van de patho-fysiologie van spasticiteit en therapeutische mogelijkheden. Vervolgens wordt een nieuw geneesmiddel beschreven, dat voor de behandeling van spasticteit ter beschikking gekomen is. Het farmacologisch profiel van dit middel, Dantroleen, riep een aantal vragen op, waar het onderzoek in het kader van dit proefschrift op is gebaseerd.
Wij onderzochten in-vitro de invloed van Dantroleen op het hart en vonden, in tegenstelling tot waarnemingen in-vivo, dat Dantroleen een lang aanhoudende, dosisafhankelijke vermindering van de contractiekracht veroorzaakte van het rattehart.
In-vivo vonden wij bij de rat, dat de kinetiek van Dantroleen verloopt volgens een twee compartimenten systeem. De discrepantie tussen de werkingsduur en de plasmaconcentraties suggereert, dat de receptor voor dantroleen niet in het centrale compartiment gelegen is.
De farmacodynamiek werd bij de mens onderzocht bij gezonde vrijwilligers en bij spastische patienten na een eenmalige orale dosis. Dantroleen veroorzaakte bij de vrijwilligers een aanzienlijke vermindering van de contraktiekracht van de musculus adductor pollicis, die gecorreleerd was aan de gemeten plasmaconcentraties. Hoewel Dantroleen vergeleken met een placebo ook een aanzienlijke vermindering van spasticiteit bij 6 van de 7 patienten veroorzaakte, was dit evenwel onder de gemeten omstandigheden niet gecorreleerd aan de concentraties van dantroleen in het bloedplasma. Wel bleek dat vermindering van spasticiteit optrad bij bloedspiegels boven 0.3 µg/ml.
In een open onderzoek gedurende 19 weken bij patienten met uiteenlopende vormen van spasticiteit, werden de bloedconcentraties na langdurige toediening bij verschillende doseringen gevolgd. Slechts bij een klein aantal patienten werd een redelijke vermindering van spasticiteit gevonden. Het aantal waargenomen bijwerkingen was groot. Er werden geen leverfunctiestoornissen gevonden.

De volgende conclusie lijkt gewettigd: Als bij een lage dagdosis (100 - 150 mg) geen duidelijk effect van dantroleen op spasticiteit wordt gezien, is het onwaarschijnlijk dat dit bij een hogere dagdosis (tot 400 mg) wel zal optreden. Positieve resultaten kwamen namelijk significant vaker voor bij patienten met een dagdosis van 100 mg Dantroleen, dan bij patienten met een hogere dagdosis. Verder steeg boven de 200 mg per dag de bloedspiegel minder dan evenredig met de dosis. Waarschijnlijk is dit terug te voeren op capaciteitsbeperkte absorptie vanuit het darmkanaal.

Op grond van dit onderzoek en dat wat inmiddels door anderen is verricht, is getracht uiteindelijk een profiel te geven aan het middel Dantroleen, anno 1978. Wij menen dat de stof - naast de mogelijkheden die het nu wellicht voor andere indikaties (maligne hyperpyrexie) lijkt te hebben - een beperkte waarde heeft bij de behandeling van spasticiteit, enerzijds, omdat de aard van de aandoening meebrengt dat lang niet alle patienten gunstig zullen reageren, anderzijds omdat het geven van het middel veel bijwerkingen ten gevolge heeft.

CHAPTER I

INTRODUCTION.

Spasticity is a pathological variation of the normal muscle tone with hyperactivity of stretch reflexes, among others resulting in an increased resistance to sudden passive movements. It is a symptom of an underlying disorder of the central nervous system and can be attended with concurrent symptoms, like paresis and other motor abnormalities dependent on the etiology and location of the lesion.

Though many efforts have been made to understand the complex disordered neuronal mechanisms, spasticity is still "a fable of a neurological demon". The facts are far outweighed by presumptions and hypotheses (Landau, 1974).

For patients spasticity is often an additional disabling factor. It frequently aggravates the existing motor deficit by involuntary muscle contractions, like flexor spasms. Such a situation asks for a reduction of spasticity usually achieved by attempts to suppress abnormal hyperactivity. On the other hand one must bear in mind that spasticity may help the paretic patient to maintain an upright position by virtue of the increased muscle tone balancing muscle weakness. It would be erroneous to reduce spasticity with therapeutic measures to such an extent that "the spastic crutches" of the patient will fail.

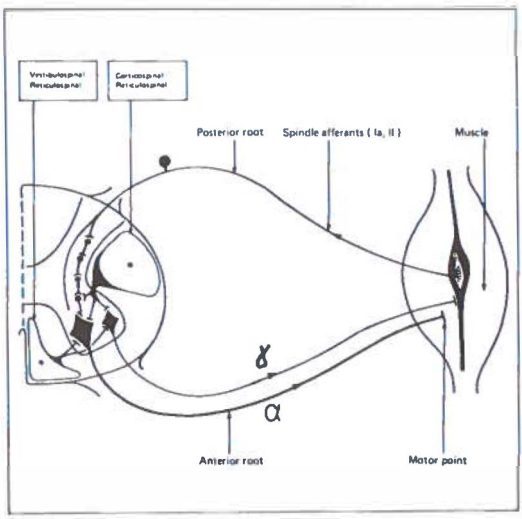
It is this controversy between the beneficial and unwanted effects of therapeutic measures in spastic patients, whose spasticity can be both helpful and disastrous to the motor function, which makes research and treatment of spasticity intriquing, satisfying, but also disappointing.

Neurophysiological considerations.

Understanding of the stretch reflex with its external and central neuronal influences has led to the concept of the regulation of motor functions. The following figure after Burke (1975) shows schematically the basic pathways that exert an influence on the stretch reflex.

This is a simple representation and may serve as a model for explanation of certain features of spasticity.

The receptors for stretch are the intrafusal muscle spindles. These are localised in the striated muscle belly, parallel to the muscle fibres.



Basic reflex pathways (simplified), after Burke.

The spindles can be considered as a group of modified muscle fibres on which, and around which afferent nerve fibres end. These afferent fibres are excitated if the muscle spindles are subjected to stretch.
Thus, because the spindles are located parallel to the muscle fibres, these will be stretched or relaxed, if the length of the muscle changes and give as a consequence information about the length of the muscle and the changes that take place. Moreover, the fibres of the muscle spindle, i.e. intrafusal fibres, themselves have the property of contraction. If the spindle fibres are shortened due to contraction, then these will be stretched to a larger extent at changes of the muscle and the afferent fibres will be more excitated.
The normal muscle fibres, i.e. extrafusal fibres, are innervated by rather large axons from the anterior horn of the spinal cord derived from the α motoneurons. The intrafusal fibres are innervated by small axons from neurons of the same region in the spinal cord, the γ motoneurons (or fusimotor neurons). The latter modulate the sensitivity of the muscle

spindles (R.E. Burke, 1972). Temperature may also influence the sensitivity of the muscle spindles (Knutsson, 1970).

In the spinal cord the information from the muscle spindles spreads over the belonging segment and in addition to other spinal areas and higher centres of the central nervous system.

The afferent axons from the muscle spindles can be divided into primary (Ia) and secondary (group II) fibres. The primary fibres excitate the α motoneurons of the stretched muscle monosynaptically, while they inhibit the α motoneurons of antagonists. The secondary fibres from both flexors and extensors facilitate, through interneurones, flexor motoneurons and inhibit extensor motoneurons. This applies to the lower limbs of spinal animals and spinal man with an isolated spinal cord, but the process may be quite different in intact animals and in normal men (Lance and McLeod, 1975).

Apart from the muscle spindles there are other receptors activated by stretch, especially the Golgi tendon organs. They are not only located in the tendons, but also in the muscle belly at the musculotendinous junctions. These Golgi organs provide a negative feedback control of muscle contractions and inhibit the stretch reflex via Ib fibres through an interneurone at spinal level.

Activation of the Golgi receptors requires a greater degree of stretch than the muscle spindles to be excitated and they serve as a defence mechanism to excessive stretch. Moreover, the α motoneurons have their own feedback i.e. Renshaw inhibitatory mechanism. This mechanism consists of cells that are excitated by collaterals of the α motoneurons and then project back to the α motoneurons (recurrent inhibition). This system also produces facilitation of the antagonistic motoneurons by inhibiting inhibitatory interneurones of these motoneurons. (This system is not shown in the figure). The spinal reflexes are subjected to very specific patterns of differential control from a supraspinal and spinal level. Both α and γ motoneurons are influenced by the descending corticospinal tracts, inhibitatory and facilitatory reticulospinal and vestibulospinal pathways (Lance and McLeod, 1975). Proprioceptive effects from the skin, joints, bladder, etc. may also influence the spinal reflexes.

The muscle tone, the state of contraction of the muscle, is regulated by the stretch reflex, which is (as has been explained) influenced by spinal

and supraspinal regulation mechanisms.

Pathophysiology of spasticity.

The term spasticity refers to a specific disorder of the muscle tone, caused by a lesion of upper motor pathways between the cerebral cortex and the lower motoneurons in the spinal cord.

The reflexes become isolated from their supraspinal inhibitatory modulation, so that the α and γ motoneurons become abnormally excitable, causing a pathological increase of muscle tone.

The increasing resistance against passive movement of the limb and the clasp-knife phenomenon are the main characteristics of spasticity. This increased resistance is caused by the abnormally excitated stretch reflexes. The clasp-knife phenomenon is caused by an increasing inhibition of motoneurons by the Golgi tendon organs in the lengthened muscle itself. After a certain degree of stretch the extensor muscles are suddenly inhibited, while there is a facilitation of the flexors.

Two forms of spasticity are usually distinguished, i.e. spinal and cerebral spasticity. Spinal spasticity is caused by a lesion below the foramen magnum. Apart from the difference in location, both forms usually differ in the mechanism of spasticity, the cause of the lesion and their therapeutic implications, though overlapping may occur. Spinal spasticity is considered as a release phenomenon, the removal of the supraspinal control. This results ultimately in a heightened excitability of the motoneurons and in a release of primitive spinal reflexes, such as the flexion withdrawal reflex. The response to all afferent inputs, either from the muscle stretch receptors or from the skin, joints, bladder etc (propriocepsis) is therefore exaggerated (Burke, 1975).

Since the spinal interneurones spread physiologically to surrounding segments, radiation of reflex activity to both synergistic and antagonistic muscles occurs. Flexor radiation often predominates over extensor radiation, so that consequently flexion reaction in spinal spasticity dominates.

Spasticity may be characterised as α or γ spasticity, since hyperexcitability of either α motoneurons or γ motoneurons is often predominant. It appears that these motoneurons react differently to local cooling of the muscle. Cooling depresses the γ motoneurons, which results in a decrease of the muscle tone, and facilitates the α motoneurons causing an increase in

spasticity (Levine, 1954; Knutsson, 1970).
Cerebral spasticity is the result of abnormal supraspinal driving of spinal reflex mechanisms either due to a loss of descending inhibition, or to uncontrolled activity in facilitatory descending pathways. The clinical feature of cerebral spasticity is dependent on the degree of impairment of facilitatory and/or inhibitatory tracts and areas in the cerebrum. If brainstem control of the primitive spinal reflexes is not altered, the tendency of flexion, as present in spinal spasticity, does not occur. Muscle spasms hardly ever present any difficulties and the generalised reflex hyperexcitability is often not as severe as in spinal spasticity (Burke, 1975).

Assessment of spasticity.

Objective measurement of spasticity is important not only for the determination of the effect and site of action of therapeutic measures, but also for the analysis of the mechanism of spasticity itself (Pedersen, 1974).
The assessment of spasticity in the course of time is treacherous, since the clinical picture is subjected to many fluctuations. It often occurs that changes take place on successive days, but even on one and the same day the picture may vary considerably. It is important to consider these factors in intrapatient comparisons such as double-blind cross-over studies. The influence of these variations can be minimised by studying large groups of patients. Interpatient comparisons may be obscured due to considerable differences in etiology and duration of the illness, age of the patient and concomittant diseases. Stratification is adviseable, but this will often bring about very small numbers of patients in each subgroup, unless many patients can be included in the study.
The types of measurements that are used in our studies will be mentioned below.

Stretch reflex.

Stimulation of the stretch reflex provides information about the level of excitability of the muscle spindles and their monosynaptically connected motor units i.e. myotatic reflex loops. This reflex is elicited manually by the tendon tap and estimated according to the following scale:

1 : no reflex activity
2 : decreased reflex activity
3 : normal reflex activity
4 : increased reflex activity
5 : extension of the reflexogenic zône.

Ashworth rating scale.

This scale is a simple, clinical classification of the resistance against passive movements that are manually performed by the investigator. The resistance of a limb is recorded according to the description of Ashworth (1964).

1 : no increase in tone.
2 : slight increase in tone giving a "catch", when the limb is moved in flexion or extension.
3 : more marked increase in tone, but the limb is easily flexed.
4 : considerable increase in tone, passive movements are difficult.
5 : limb is rigid in flexion or extension.

RED-meter.

The RED-meter is a mechanical device that measures ("absorbs") the force that the examiner has to impose on the spastic limb in order to move this limb against the pathological resistance. Since this force, registered by the device, is equivalent to the resistance (= damping) that is offered by the spastic limb, the instrument works as an equivalent - damping (= ED) - meter. Though the degree of hypertonia may not differ between patients, variation in muscle strength may cause great differences in the degree of passive resistance. Muscle strength is related to muscle mass and the latter (roughly) to body weight. Therefore the relative equivalent damping (ED over body weight) is used and the instrument is called "Relative Equivalent Damping"(= RED)-meter (Kwee et al, 1976).
In 20 patients, who suffered from spasticity in varying degrees, we compared the RED-meter and the Ashworth rating scale. The values obtained with both methods were found to correlate fairly well with the ranking-order of severity of spasticity that was given to these patients by their attending physician, an experienced rehabilitation-doctor. Due to its very nature, however, the RED method will discriminate better between patients.

The test with the RED-meter is performed in patients lying on a bed in a relaxed position with their lower legs hanging down at an angle of 90^{o} with the thigh. The RED-meter is attached just above the ankle of the lower leg. The lower leg is swung around the kneejoint between 60^{o} and 120^{o} during 10 strikes at a constant velocity. The test is repeated at different frequencies (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 Hz) with intervals of 1 minute. The RED values of the first 10 strikes are added up. Because of a characteristic frequency dependency, the ultimate value is the average of the RED at different frequencies.

ADL.

This scale assesses the activities of daily life of the patient. In our study we took into consideration dressing, moving and personal hygiene as activities of daily life. The assessment is influenced by possible concurrent deficits not due to spasticity. Furthermore it does not discriminate between drug- and non-drug related changes in spasticity; for instance between spontaneous declining of the condition and side effects, such as muscular hypotony, which is observed with most muscle relaxants.

On the whole it is a method that does not measure spasticity as such to a very specific degree, but it does measure the goal of the therapy, which seems to be of greater importance.

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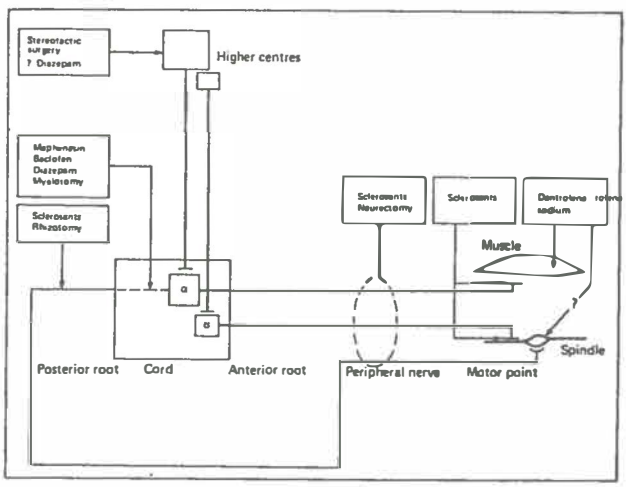
CHAPTER II

THERAPEUTIC METHODS.

Management of the patient with spasticity implies more than just treatment of spasticity. Reduction of muscle tone may not be in the patient's interest, particularly in those cases where the abolition of spastic stiffness unmasks severe muscle weakness. The increased muscle tone is not harmful and may even be beneficial for those paretic muscles that keep the patient in the upright position (Burke, 1975). Therapeutic methods may act at different sites of the reflex arch and its related centres and tracts. If no satisfactory results are obtained with a given measure, replacement by or combination with a measure that acts on a different site of the reflex arch is to be considered. The figure below after Burke (1975) shows the probable sites of therapeutic action; the various methods will be discussed below.



Probable sites of therapeutic action, modified after Burke.

Surgical methods.

These methods are destructive, definite procedures, which will only be used

if no other treatment can relieve the patient's very severe, disabling hypertonia. As these measures are in fact beyond the scope of this thesis, only a brief review is given:

Tenotomy - for the treatment of isolated contractures, for instance of the calf muscle and the hamstrings. Appropriate tendon transfer operations may benefit individual patients.

Stereotactic lesions - in the basal ganglia and in the nuclei and efferent pathways of the cerebellum. This measure is restricted to very severe cerebral spasticity to correct the imbalance between descending facilitatory and inhibitory pathways.

Interruption of the afferent side of the reflex arch-like neurectomy and posterior rhizotomy (both outside the spinal cord) or myelotomy (within the spinal cord). These measures are possibilities in spinal spasticity, but also in very severe cerebral spasticity (Heimburger et al, 1973). Often the muscle tone is only temporarily reduced.

Neurolytic agents.

A number of the surgical measures can be replaced by injections with neurolytic agents, which cause neuronal destructions, like alcohol 40-50% and phenol 2-5% (Khalili et al, 1964). These agents can be injected into the lumbar theca, into the peripheral nerve, or into the motor points of the spastic muscles. The duration of the effect has been reported to vary over a considerable range from 10 to 850 days (Khalili et al, 1964; Delateur, 1972).

Sclerosants however are not very selective in their action, so that at each side both sensory and motor fibers will be affected, though there is a preference for the thin fibers i.e. gamma fibers.

Since unwanted side effects, for example paraesthesias may occur, (which often disappear in several days or weeks), Awad (1972) suggested to block only pure motor nerves such as the obturator nerve, in order to avoid paraesthesias.

Low lumbar injections have the great disadvantage to affect also the nerves that innervate the bladder and sphincter ani.

The exact location for injection with sclerosants can be found by testing with a local anaesthetic first. The motor points can be found by stimulation of the muscle via a needle. Phenol blockade of the motor points is a useful technique in the treatment of localised spasticity, such as hypertonia of the tibial muscle.

Pharmacological agents.

There are several muscle relaxants that act at different sites of the reflex arch, connecting pathways and areas. Often the therapy is based on "trial and error". This emphasises an objective assessment of spasticity. It should be noticed that most patients with chronical neurological disorders are very susceptible to placebo effects. Any drug efficacy study should therefore strictly be controlled to eliminate these effects, as well as those due to observer bias.

Muscle relaxants can be divided into drugs that depress the central nervous system with preference for the polysynaptic pathways, and more peripheral acting drugs that exert their action in the endplate or in the muscle itself. To the last group the neuromuscular blocking agents belong, which, although widely used in anaesthesiology, are ineffective in controlling spasticity in doses that do not cause paralysis. The muscle relaxants generally used for the treatment of spasticity are discussed below.

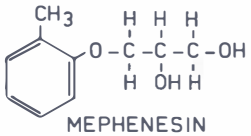
Mephenesin and similarly acting compounds.

In 1946 Mephenesin, 3-(O-methylphenoxyl)1,2-propanediol was found to cause reversible paralysis in animals without obvious gross sedation (Berger and Bradley, 1946).

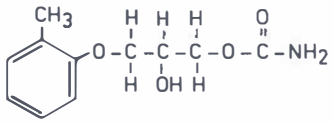
The drug acts selectively on the polysynaptic pathways, i.e. the interneurones in the spinal cord. It antagonises the action of strychnine, which reverses the polysynaptic inhibition of the naturally transmitter glycine.

The clinical enthousiasm clearly outweighed the therapeutic benefit (Domino, 1974). Due to its lack of potency, high doses of mephenesin are needed to produce muscle relaxant activity. Moreover, its action is of limited duration, because of a very short half life. Mephenesin is so quickly biotransformed, that effective bloodlevels are not reached after oral administration. Intravenously given it only has muscle relaxant properties in doses that can cause red cell hemolysis. These properties made the drug clinically not useful.

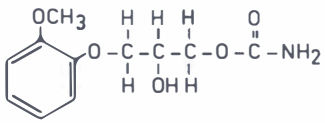
Several other compounds have been found to act in a similar way like mephenesin, but are relatively long-acting (Roszkowsky, 1960). Derivatives of mephenesin like mephenesincarbamate and methocarbamol have a longer half life (Truitt and Little, 1958), but are still not potent enough for clinical use (O'Doherty and Shields, 1958).



MEPHENESIN

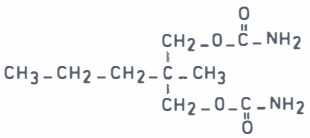


MEPHENESIN CARBAMATE

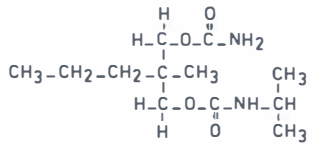


METHOCARBAMOL

Mephenesin and its derivatives

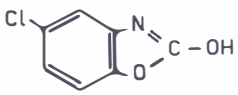


MEPROBAMATE



CARISOPRODOL

Carbamate derivatives



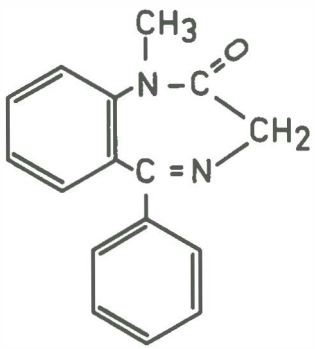
CHLORZOXAZONE

Benzazole compound

The carbamate derivatives meprobamate and carisoprodol also resemble mephenesin in their muscle relaxant activity. They are not only not potent enough, but in addition they cause much more sedation. In one study, carisoprodol caused 30% side effects, chiefly vertigo and drowsiness (L. Meyler and Herxheimer, 1972).

Another compound with the same pharmacologic properties, the benzazole compound chlorzoxazone is also too weak to be effective in clinical practice. Chlorzoxazone is usually combined with an analgesic and prescribed for relief of pain associated with acute muscle spasms (Ogden and Schockett, 1960). The effectiveness on for example low back pain is probably only caused by the analgesic agent in the combination.

Diazepam.



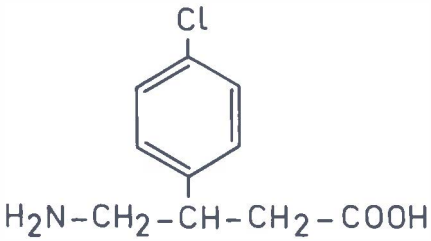
DIAZEPAM

This benzodiazepine is also classified as a centrally acting muscle relaxant, which suppresses the activity in the spinal reflex pathways (Lapierre et al , 1973), mainly the polysynaptic pathways, the interneurones in spinal cord and brainstem. It acts primarily on the flexor reflex, but also on the extensors, though in higher concentrations (Herman, 1974). Thus diazepam is of benefit in spinal spasticity and of little value in cerebral spasticity. Benzodiazepines also suppress neuronal activity in the reticular formation, which on the one hand contributes to the muscle relaxation, but on the other hand causes the main unwanted effect, namely sedation.

Recent investigations by Verrier (1976) show a peripheral action of diazepam on the neuromuscular apparatus. 15-30 mg given intravenously reduces significantly the amplitude of the compound action potential of the direct muscle response (M wave), and (maybe as result or as concurrent effect) decreases the isometric twitch tension.

Baclofen.

Baclofen, beta(4-chlorophenyl) gamma-aminobutyric acid, is a lypophilic substituent of GABA (Keberle and Faigle, 1972), which can penetrate



BACLOFEN

the bloodbrain barrier after oral ingestion. Saito (1975) showed that it antagonises substance P., which is supposed to be an excitatory transmitter of the primary afferent synaps in the spinal cord.
Baclofen depresses both monosynaptic and polysynaptic transmission (Bein, 1972), but has no influence on the neuromuscular transmission. Predominantly spinal sites of action seem likely, since in animal experiments reflex inhibition after decerebration was equal to the inhibition after spinalisation. It is suggested that baclofen acts predominantly on the polysynaptic pathways. (Burke et al, 1971).
Baclofen decreases preferentially gamma spasticity in patients, who are characterised as being cryopositive by local cooling (Knutsson et al, 1973).
The drug is well absorbed after oral administration with blood peaklevels about 2 hours after absorption. The mean half life is 3-4 hours. It is excreted unchanged in the urine and faeces at a total amount of 85%, while only a small part is metabolised in the liver by de-amination (Keberle and Faigle, 1972).
In human experimental studies (Levine et al, 1972) baclofen caused spasmolytic effects with a decreased electrical (EMG) response of the muscle of more than 30%. The maximum effective dose varied considerably in this group of 5 patients (15-80 mg per day).
Knutsson et al, (1972) found a reduction of the mean amplitude of the tendon jerks of 10 to 35% after baclofen 0.2 mg/kg i.v.
In double-blind trials baclofen 30 to 80 mg daily has been reported to be superior to placebo in relieving spasticity by Jerusalem (1968), Pedersen et al, (1970), Hudgson and Weightman (1971) and Duncan et al, (1976), while Ketelaer and Ketelaer (1972) considered baclofen superior

to diazepam in comparable doses, but their study was not done under double-blind conditions. From and Heltberg (1975) found baclofen to be equally effective as diazepam, but with less unwanted sedative effects. The main side effects are muscular weakness, tiredness and nausea, which can be minimised by adjusting the dose slowly.

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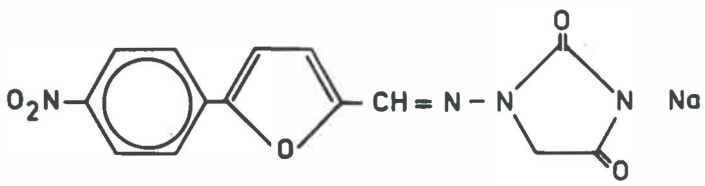
CHAPTER III

DANTROLENE SODIUM.

Dantrolene sodium is the latest development in the pharmacological treatment of spasticity. In the United States of America this drug has been available since 1974 (de Haen, 1974). In the Netherlands it was registered in 1977.
Dantrolene sodium has a unique place among the muscle relaxants that are in use for the treatment of spasticity, because its site of action is not within the nervous system, but in the muscle itself. Moreover there is experimental evidence that dantrolene sodium may be a therapeutic agent not only for the treatment of spasticity, but also against malignant hyperpyrexia (Harrison, 1975).
In this chapter the profile of dantrolene sodium is described, as it was known to us at the moment we became interested in this drug.



Dantrolene sodium

Dantrolene sodium, 5-(p-nitrophenyl)furfurylidene-amino-hydantoin sodium is a hydantoin derivative (mol. weight: 336) (Snyder et al, 1967). Ellis and Carpenter (1974) showed that dantrolene sodium decreases

the Straub-tail phenomenon caused by morphine in mice, without loss of the righting reflex or motorcoordination. This effect is rather specific for dantrolene sodium, in contrast with the effect seen after administration of central acting muscle relaxants like mephenesin and diazepam.

The drug causes a dose-dependent long-lasting decrease of spontaneous motoractivity and muscle tone with no decrease of muscle coordination on the rotator rod and no marked sedation in mice, while no local anaesthetic action was observed (Ellis et al, 1973). Dantrolene sodium causes marked reduction of rigidity in decerebrated cats, but unlike the central acting muscle relaxants it has no preferential effect on polysynaptic reflex responses (Ellis at al, 1973).

In vitro it has no effect on the electrical properties of the nerve and neuromuscular transmission (Lowndes, 1975; Kurihara and Brooks, 1975). It does not affect the electrical excitability of the muscle membrane. This was found in the amphibian skeletal muscle in-vitro (Ellis and Bryant, 1972) and in-vivo (Nott and Bowman, 1974). Dantrolene sodium raises the threshold for caffeine induced contractures and markedly depresses potassium induced contractures (Ellis and Carpenter, 1972; Putney and Bianchi, 1974). In twitch experiments on the isolated frog sartorius muscle, Putney and Bianchi (1974) found a significantly decreased influx of labelled calcium whereas the resting calcium influx was not affected. They suggested that dantrolene sodium affects the triggering step in the excitation-contraction coupling by decreasing the calcium efflux from the sarcoplasmatic reticulum.

Hainaut and Desmedt (1974) injected barnacle single muscle fibers with calcium sensitive biolumnescent aequorin, which binds to the intracellular ionised calcium. They found a reduction of the intracellular calcium, suggesting that dantrolene sodium acts on the intracellular storage sites and reduces the free calcium.

Brocklehurst (1975) showed in the "skinned" preparation of frog muscle (i.e. without membrane), which contracts with a calcium containing gel, that dantrolene sodium acts on the calcium release and not on the re-uptake in the sarcoplasmatic reticulum.

Based on these pharmacologic data our first question was: if dantrolene sodium affects the calcium release in the sarcoplasmatic reticulum in skeletal muscle, will this also happen in the sarcoplasmatic reticulum

of other muscle types, for instance cardiac muscle? If so, does dantrolene sodium also have an effect on the contractile activity of the heart? In Ch. IV in-vitro experiments in which we tried to give an answer to this question are described.

In animal studies, a dose-dependent relaxation by dantrolene sodium appears to be mainly found on the twitch tension, while the tetanus is less affected. Ellis and Carpenter (1972) found a twitch/tetanus decrease ratio of 2.4 - 3.6 in the sartorius muscle of the frog. Lowndes (1975) showed in the cat soleus muscle, that the dose-dependent depression of the indirectly elicited contractile strength is more pronounced at lower frequencies of stimulation. And again the tetanus is affected to a lesser degree. Also Nott and Bowman (1974) found a twitch depression after both direct and indirect stimulation in cats; the m. tibialis anterior (fast contracting muscle) was slightly more affected than the m. soleus (slow contracting muscle). The tetanus is much less depressed, with the same difference between the two types of muscles. These differences between slow and fast contracting muscles were also found by Monster et al, (1974), who compared the m. soleus and m. gastrocnemius in decerebrated cats.

The results of in-vitro studies cannot be extrapolated to in-vivo situations without critical comment. First: it depends on the question whether the in-vitro concentrations, at which certain effects are found, can be obtained at all in in-vivo situations. Second: due to pharmacokinetics, drug effects in the intact animal will be different from the effects in-vitro. Third: in-vivo, active metabolites may be responsible for the effects. Dantrolene sodium is metabolised in the liver mainly by 5-hydroxylation of the hydantoin group and for a small part by reduction of the nitrogroup, followed by acetylation (Hollifield and Conklin, 1973; Lietman et al, 1974). Regarding these considerations, we investigated in the following study in rats the relationship between concentrations of dantrolene sodium in plasma and muscle tissue and the effect on the skeletal muscle (Ch. V).

In man dantrolene sodium reduces muscle contractility and reflex activity without noticeable effect on the voluntary muscle strength. Monster et al, (1974) found in 18 normal volunteers who received dantrolene sodium 100-125 mg orally, peak bloodlevels of 1.2 - 1.7 µg/ml after 3 - 6 hours. They showed a reduction of muscle contractility, which

was dependent on the frequency of the electrical stimulation and on the muscle length.

Herman (1972) investigated normal, hemiplegic, and paraplegic subjects. In 5 patients dantrolene sodium 125 - 150 mg orally gave a 40 - 70 % reduction of the ankle torque, induced by Achilles tendon taps, after 1½ - 3 hours. This reduction was not attended by a comparable decrease of electromyographic activity. The ratio between torque reduction and integrated E.M.G. increased 1½ - 3 fold, suggesting that dantrolene sodium has no effect on muscle excitability. The isometric twitch tension was reduced by 48 - 60 % of pre-drug levels 1½ - 3 hours after ingestion of the drug. These authors also observed a discrepancy between low and high frequency stimulation. In case reports 2 subjects showed reasonably constant voluntary muscle strength, but the integrated E.M.G. revealed an increase in electromyographic activity. In one subject, however, the voluntary muscle strength decreased with 25% without changes in E.M.G. activity.

In patients Monster (1974) found a dose-dependent reduction of the tendon tap and clonus, which was not consistently parallelled by functional improvement, though those patients who had a functional improvement, showed decrease of reflex activity. In another study Monster et al, (1974) found a correlation between dose level and percentage reduction of the twitch tension after stimulation of the tibial nerve. This reduction was more pronounced at shorter muscle length (dorsiflexion). The voluntary muscle strength was significantly less reduced than the twitch tension. Six normal subjects showed peak blood levels ranging from 0.7 to 1.45 µg/ml after 100 mg dantrolene sodium orally. Clinical effects of the drug were not mentioned.

Former studies show that dantrolene sodium causes a decrease of different muscle activity parameters, whereas information about the relationship between blood levels and effect is scanty. In the papers mentioned, this relationship is reported in detail only for a few, single subjects. Therefore our next part of research was concerned with the relationship between plasma levels of dantrolene sodium and the effect on the twitch tension in normal subjects on the one hand, and between plasma levels and the effect on hypertonia in spastic patients on the other hand. The results of this study are decribed in Ch. VI.

Dantrolene sodium was studied clinically in several double-blind and open studies to establish the efficacy on spasticity of various origin. Chyatte and Birdsong (1971) investigated 45 patients in a double-blind trial during 4 weeks. The dosages of dantrolene sodium ranged from 25 to 400 mg daily. They found that reflex activity and resistance against passive stretch decreased, whereas the amplitude of active movements in affected limbs increased significantly, compared with placebo treatment.

Dantrolene sodium was preferred to placebo in its effect on spasticity in 9 hemiplegic patients in a placebo controlled study (Chyatte et al, 1974). Each drug was given for 11 days, the dosage of dantrolene sodium being 50 to 75 mg t.i.d., with a maximum of 300 mg daily.

In a multicentre placebo controlled trial, 147 patients with spasticity of different origin were analysed (Monster, 1974). In 68% of the cases the optimum dose of dantrolene sodium was 400 mg per day. Reflex activity and clonus improved during the drug period, though this did not always mean a clinical improvement as well. Passive resistance did not seem to be affected by dantrolene sodium. The "overall clinical response" was improved in a significant proportion of cases. This appeared to be due to the reduction of clonus and involuntary movements; the activities of daily life (ADL) also improved, although to a much lesser degree than the "overall clinical response" (43% versus 83%).

Chipman et al, (1974) examined 13 patients in a double-blind cross-over trial versus placebo during 2 periods of 5 weeks. On dantrolene sodium, at a dose ranging from 200 to 400 mg per day, 11 patients improved. Severity of clonus and activity of the tendon jerks changed most markedly. The authors found no difference in reaction between cerebral and spinal spasticity.

Chyatte et al, (1973) investigated the effect of dantrolene sodium and placebo in 17 patients with cerebral palsy in a double-blind cross-over manner. The patients took dantrolene sodium up to 400 mg daily and placebo during 4 weeks each; 12 patients continued the medication after the trial, since the treatment was succesful. After 4 months, however, 4 more patients dropped from the treatment, because improvement was minimal.

Sheplan and Ishmael (1975) found dantrolene sodium to be better than placebo in a double-blind cross-over trial in 18 patients during 10 weeks.

The maximum dosage was 800 mg daily. Reflexactivity was decreased with more than 40%. There was a marked clinical improvement.
Haslam et al, (1974) investigated the effect of dantrolene sodium in 23 children with cerebral palsy (1½ - 17 years of age) in a double-blind cross-over trial. Each drug period was 15 days, and the maximum dosage was 12 mg/kg/day. Dantrolene sodium decreased reflexactivity (except clonus) and "scissoring" better than placebo. The authors found significant changes, which showed no consistent pattern, for different parameters between boys and girls. The drug improved the results of occupational therapy significantly, but had no influence on physical therapy or nursing evaluations.
In 28 children with cerebral palsy, who were investigated in a double-blind cross-over study by Denhoff (1975), dantrolene sodium appeared to improve the condition of 14 children during 6 weeks of treatment.
Dantrolene sodium in Multiple Sclerosis was found less succesful; in a doubleblind cross-over study (Gelenberg and Poskanzer, 1973) only in 6 out of 20 patients dantrolene sodium (up to 800 mg per day) was better than placebo. Major side effects were muscle weakness and nausea.
Identical results were obtained by Tolosa and Soll (1975). They found in 23 patients with moderate to severe Multiple Sclerosis, with 12 patients on the active substance up to 800 mg daily, only reduction of spasticity in 5 of these patients and in 3 patients on placebo. Numerous and disturbing side effects were noticed; muscular weakness in 6 patients, moreover dizziness, vertigo and gastro-intestinal complaints.
In an open trial Jonnson (1975) treated 11 hemiplegic patients with dantrolene sodium up to 400 mg per day. Spasticity decreased, however without a meaningful concurrent increase in ADL functions. In some cases medication had to be discontinued due to marked tiredness. The positive effect seemed to be only temporarily, while only in a few patients spasticity increased, if medication was discontinued.
Steinberg and Ferguson (1975) treated 23 hemiplegic patients. Dantrolene sodium at a maximal dosage of 600 mg per day showed improvement in motor performance and was most effective against clonus, but less effective against passive stretch and tendon reflexes. There was a significant difference in response between people older and younger than 50 years; the latter responded best.

The former mentioned reports on therapeutic usefulness are sometimes conflicting and notoriously overshadowed by possible drug related side effects and toxic reactions. Abnormalities in bloodchemistry have been observed, but these have largely been transient (Chyatte and Birdsong, 1971; Chyatte and Basmayan, 1973; Gelenberg and Poskanzer, 1973; Mayer et al, 1973). Isolated cases of fatal hepatitis have been reported in adults taking the drug for 60 days or longer (F.D.A., 1975). In children, increased enzyme levels or other signs of liver disorders were remarkably rare (Haslam et al, 1974). A causal relationship between dantrolene sodium administration and liver dysfunction has not been established, though it can not be excluded.
From the studies mentioned above, a definite effect of dantrolene sodium in various spastic conditions seems likely. On the other hand it is not known generally in how far these results are correlated with plasma levels, and whether failures may be due to insufficient plasma levels. Neither is it known whether toxic symptoms, if they appear, are consistently related to them. Both questions are not easy to answer. In an open clinical trial we tried to make an attempt. The results of this study are presented in Ch. VII.

In summary, the pharmacological data of dantrolene sodium left us with some questions.
Ch. IV. Dantrolene sodium depresses the contractility of skeletal muscle, possibly by interfering with the calcium release from the sarcoplasmatic reticulum. We investigated the effect of dantrolene sodium on the isolated rat heart and compared the results with the effect on the rat diaphragm.
Ch. V. In how far results of in-vitro studies with dantrolene sodium were relevant for in-vivo situations was subject of the following experiments We investigated the relationship between concentrations of dantrolene sodium in plasma, skeletal muscle, and heart muscle and the effect of dantrolene sodium on the m. tibialis anterior of the rat.
Ch. VI. Whether the animal results presented in the former chapter were applicable to human situations was the next question. Therefore we investigated the relationship between plasma levels of dantrolene sodium (and its major metabolite, 5-hydroxy dantrolene) and the effect on the twitch tension in normal subjects, and the effect on hypertonia in spastic patients after a single dose of 100 mg.

Ch. VII. The efficacy of dantrolene sodium in the treatment of spasticity is obvious, though many conflicting data arise from the trials reported. In an open trial we investigated patients with spasticity of different origin and correlated the concentrations of dantrolene sodium and its major metabolite in plasma with the results on spasticity after prolonged administration. Special attention was paid to possible adverse reactions of the drug.
In Ch. VIII. the different studies are discussed together.

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CHAPTER IV

THE EFFECTS OF DANTROLENE SODIUM ON CARDIAC AND SKELETAL MUSCLE IN RATS.[1]

1. Introduction

Dantrolene sodium is a muscle relaxant, which acts by depressing the contractility of striated muscle. It inhibits the excitation-contraction coupling, possibly by interfering with the calcium release from the sarcoplasmatic reticulum.

Fabiato and Fabiato (1973) and Trautwein (1973) suggest that the sarcoplasmatic reticulum of the heart muscle also plays an essential role in the variable calcium release and uptake in excitation-contraction coupling. This could mean that dantrolene sodium has an effect on cardiac contractile activity as well.

Ellis and Simpson (1975) found under basal conditions no effect in dogs on cardiac parameters such as cardiac output, frequency, ECG, and blood pressure. The latter investigation was done on intact animals, so that relatively small changes in cardiac output and blood pressure may have been masked by compensating mechanisms of the cardiovascular system.

Therefore we investigated the activity of dantrolene sodium on frequency and contractility of rat cardiac muscle in vitro. The influence on contractility was compared with that on the skeletal muscle (i.e. the rat diaphragm) in vitro.

2. Materials and methods

Female white rats, 35-40 weeks old and weighing 200-250 g were used in all experiments.

2.1. The isolated heart

After decapitation of the animal the chest was opened, the aorta was quickly cannulated with a glass cannula (Ø 2.5 mm) and the heart was

1. This chapter has been published (except for some slight modifications) in the European Journal of Pharmacology, 1976, 39, 127 (Willem J. Meyler, Harry Wesseling and Sandor Agoston).

removed and placed in a modified Langendorff apparatus (Langendorff, 1895).

The coronary arteries were perfused with Meyler's solution (NaCl 128 mM, KCl 4.7 mM, $CaCl_2$ 1.3mM, NaH_2PO_4 0.4 mM, $MgCl_2$ 1.0 mM, glucose 10 mM), with Hepes buffer (10 mM) and oxygenated with 95% O_2-5% CO_2.

Hepes buffer has been shown to be without significant effect on cardiac muscle contractility, if physiological sodium and calcium concentrations are used (Dresel, 1974). The temperature was kept constant at 37°C, and pH was adjusted to 7.4 with NaOH. Heartrate and contractility were maintained by a continuous adrenaline infusion (adrenaline 4.1×10^{-5} M. flow rate 3.30 ml/h) into the perfusion fluid.

The contractility of the spontaneously beating heart was continuously measured by means of a Hottinger force-displacement transducer, amplified by a Hottinger amplifier and recorded on a Polygraph 34-recorder. Heart rate was measured after 3, 10, 20 and 30 min on a Tektronix 2B 67 storage oscilloscope. An equilibration period of half an hour was allowed for each preparation, after which base line values of heart rate and contractility were determined; these were considered to be 100% values. If no constant base line could be achieved, the preparation was rejected.

Dantrolene sodium solutions were prepared so as to give the following concentrations in the perfusion fluid: 5.5×10^{-6} M(1.87 mg/l), 1.1×10^{-5} M (3.75 mg/l), 2.2×10^{-5} M (7.5 mg/l) and 4.4×10^{-5} M (15 mg/l) the last concentration being approximately saturated.

Contractility and frequency were measured after 3, 10, 20 and 30 min,. after which the heart was perfused again with Meyler solution without dantrolene sodium during another half hour. Since control values were not restored during this period, only one concentration of dantrolene sodium was used in each experiment.

2.2. Rat diaphragm muscle-phrenic nerve preparation

After decapitation of the rat, the chest was opened and the left part of the diaphragm with attached phrenic nerve was removed according to Bülbring (1946), and bathed in Meyler solution with Hepes 10 mM, and oxygenated with 95% O_2-5% CO_2 after adjustment to pH 7.4 with NaOH. The temperature was kept constant at 37°C. The composition of the bathing fluid was thus the same as in the isolated heart experiments.

We did another series of experiments on rat diaphragma, but used Krebs solution which has a Calcium concentration of 2.5 mM.
The phrenic nerve was stimulated with supramaximal square wave stimuli at a frequency of 0.1 Hz with 0.3 msec duration, delivered by a Grass stimulator. The isometric twitch tension was recorded by a Hottinger force-displacement transducer, amplified by a Hottinger amplifier and registered continuously by a Riker Denshi recorder.
After a one hour equilibration period, dantrolene sodium in the following concentrations, dissolved in the bathing medium, was administered for 30 min: 3.4×10^{-7}M (0.12 mg/l), 5.5×10^{-6}M (1.87 mg/l), and 4.4×10^{-5}M (15 mg/l). After this the muscle was washed out and put back in Meyler c.q. Krebs solution without dantrolene sodium. A new preparation was used for each concentration.
The results were analysed statistically using Student's t-test.

3. Results

Dantrolene sodium gave a dose-dependent reduction of the contractility of the isolated rat heart; this reduction amounted to 76%, when the maximum concentration of 4.4×10^{-5}M (15 mg/l) was administered (fig. A concentration of 2.4×10^{-5}M (8.0 µg/ml) caused a 50% reduction of contractility. Dantrolene sodium had no effect on the heart rate (table 1) in any of the concentrations used.
The drug also decreased contractility of the rat diaphragm in vitro; there seemed to be a small difference between the dose response curves in 1.3 and in 2.5 mM CaCl (fig. 1), though the effects of corresponding concentrations did not differ significantly. A drug concentration of 1.9×10^{-6}M in Meyler and 4.7×10^{-6}M in Krebs caused a 50% reduction. Just as in the cardiac muscle, contractility would not be restored within the observation period for any concentration used. Maximal concentrations of 4.4×10^{-5}M (15 mg/l) gave a reduction of 74% of control values (fig. 1). The dose response curves were however considerably less steep than in the heart preparation; they had about the same slope as was found by Ellis and Carpenter (1972).
This implies, that rat skeletal muscle is more sensitive to dantrolene sodium in low concentrations than rat cardiac muscle. When the same calcium concentrations are compared, a ratio of about 10 was found for the doses that decreased contractility by 50%; if the calcium concen-

DS conc (M) in Meyler's solution	Heart rate			Contractility
	mean control values (± SEM)	after ½ h. incubation	% change in heart rate	% change
0	301 ± 18.0	280 ± 30.3	- 7	+ 3
5.5×10^{-6} M	297 ± 23.0	269 ± 16.2	-10	+12
1.1×10^{-5} M	272 ± 8.0	280 ± 28.9	+ 3	-18*
2.2×10^{-5} M	274 ± 16.3	282 ± 9.1	+ 3	-44*
4.4×10^{-5} M	274 ± 5.3	260 ± 17.5	- 4	-73*

Table 1: The influence of dantrolene sodium (DS) on frequency and contractility of the isolated rat heart. There are no significant differences between heart rates.
*: Significantly ($p < 0.05$) different from preceding value.

tration was twice as high, the ratio was 5. This ratio is of little significance for the difference in sensitivity between both types of muscle, since the dose-response curves were not parallel.

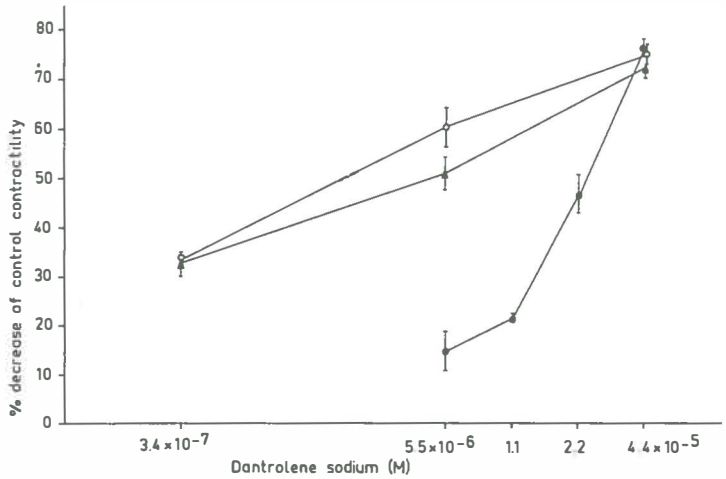

Fig. 1: Decrease of contractility of the isolated rat heart and diaphragm with various concentrations of dantrolene sodium measured as percent (± SEM) of pre-drug control values.
● — ● rat heart in Meyler perfusion fluid
o — o diaphragm in Meyler perfusion fluid
▲ — ▲ diaphragm in Krebs perfusion fluid
Each point represents 3 determinations.

4. Discussion

Our results indicate that dantrolene sodium is not only active on skeletal muscle, but also on mammalian cardiac muscle; in the latter case in higher concentrations. Since the drug is claimed to relieve spasticity, long-term use will frequently be necessary. Only a few estimations of plasma concentrations have been reported from human studies. Herman and Mayer (1972) found plasma levels between approximately 2.5-3.5 µg/ml after 100 mg dantrolene sodium 4 times daily for several weeks in four paraplegic patients. The authors did not describe a concentration effect relation in these patients.
Though our findings cannot be extrapolated to clinical situations, it should be born in mind that the concentration giving 50% inhibition

of cardiac muscle contractility was only about 2-3 times higher (8.0 µg /ml) than the one reported in human studies. A maximal dantrolene sodium dosage of up to 800 mg daily has been described (Tolosa and Soll, 1975).

Our observations that dantrolene sodium decreases the contractility of the spontaneously beating rat heart in vitro, were confirmed by Bowman and Khan (1977) in studies on isolated guinea-pig atria. Others however, (Ellis et al, 1973; 1976; Butterfield and Ellis, 1973; Ellis et al, 1975 and Harrison, 1975) found no significant changes in myocardial contractility in different species. It should be noticed, that the former experiments were done on intact animals, while bloodlevels of dantrolene sodium were not reported. Possible effects of dantrolene sodium on the unloaded heart in these experiments may have been masked by compensatory mechanisms of the cardiovascular system. It is not impossible, that under increased labour the drug will affect myocardial contractility and thus reduce cardiac output even after one single dose.

In in-vitro experiments van Winkle (1976) observed that the calcium binding and release phase of the cardiac muscle sarcoplasmatic reticulum were not affected in contrast with skeletal muscle sarcoplasmatic reticulum, whereas Ellis et al, (1976) using atrial strips observed an effect on contractility, although they used much higher concentrations of dantrolene sodium than necessary for skeletal muscle relaxation. Though dantrolene sodium is not known to have induced heart failures under clinical conditions, the effects of this compound in animals and man on both skeletal and cardiac muscle in relation to bloodlevels and to exercise should be investigated further.

Since dantrolene sodium is supposed to inhibit mainly calcium release from the sarcoplasmatic reticulum (Nott and Bowman, 1975; Putney and Bianchi, 1974) this may explain the fact that changes in external calcium concentrations are not very critical in skeletal muscle.

The suggested mechanism of action of dantrolene sodium might also be the reason why, in either kind of experiment, no complete depression of contractility could be reached, but only a decrease of approximately 75% at 15 mg/l. Another explanation might be that contractility could only be depressed to a greater extent with concentrations higher than 15 mg/l. As in several other experiments (Ellis and Carpenter, 1972;

Ellis and Bryant, 1972) 15 mg/l was the highest concentration of dantrolene sodium that we could obtain. On the other hand, a similar maximal inhibition of twitch tension in indirectly stimulated rat hemidiaphragm-nerve preparation was reported after exposure to 30 mg/l concentration of dantrolene sodium by Kurihara and Brooks (1975). Therefore it is unlikely that higher concentrations of this compound would further increase muscular blocking activity.

Ellis and Bryant (1972), and Putney and Bianchi (1974) found that dantrolene sodium did not affect the electrical properties of the muscle membrane, i.e. the action potential. This explains why the decrease in cardiac contractility was not parallelled by a drop of the heart rate. Therefore the effects of dantrolene sodium on the cardiovascular system cannot be assessed by heart rate and E.C.G. only.

In summary we may conclude that our experiments in vitro confirm the previous findings that dantrolene sodium, in low concentrations, does depress the contractility of skeletal muscle. With higher concentrations however, cardiac muscle is affected as well.

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CHAPTER V

THE EFFECT OF DANTROLENE SODIUM ON RAT SKELETAL MUSCLE IN RELATION TO THE PLASMACONCENTRATIONS.[1]

1. Introduction.

In this study we investigated the relationship between the muscle relaxant effect on the skeletal muscle, and both plasma and tissue concentrations of dantrolene sodium following intravenous and oral administration in the rat. Moreover we determined the disappearance of the drug from the plasma and tissue after intravenous injection.

2. Material and methods.

Male white Wistar rats of 200 - 300 g weight were used. All experiments in which muscle contractions were measured, were carried out under pentobarbital anaesthesia. The rats were artificially ventilated with air. Bloodpressure was monitored continuously with a catheter inserted in the external carotid artery. Rectal temperature was kept constant at 37^{o}- 38^{o} C by using a heating lamp. Drugs were administered either intravenously by injection into the external jugular vein or orally by a tube into the stomach. Both intravenous and oral solutions contained 5 mg/ml dantrolene sodium dissolved in propyleneglycol: aqua dest. = 9:1 at pH 12 (Nott and Bowman, 1974).

2.1. Dose-(maximal)response experiments.

Dantrolene sodium was administered intravenously by bolus injections of 1, 2 and 4 mg/kg.

The isometric twitch tension of the left tibialis anterior muscle, elicited by indirect supramaximal square wave stimulation of 0.3 ms duration at a rate of 0.2 Hz by a Grass stimulator, was continuously measured with a Hottinger-Baldwin force displacement transducer, amplified by a Hottinger amplifier and registered by a Riker Denshi recorder. Since twitch depressions did not return to normal within hours, only

1. This chapter has been accepted for publication in the European Journal of Pharmacology.

one dose of dantrolene sodium was used in each experiment.

2.2. Plasmaconcentration-effect experiments.

In one series of experiments, where dantrolene sodium 2 mg/kg was injected, rats were decapitated and blood samples were collected at the time of maximal twitch depression, and at 60%, 75% and 90% recovery to the initial constant twitch tension.

In another series dantrolene sodium 25 mg/kg was administered orally; the rats were sacrificed at the time when the maximal block was reached and then blood was collected.

2.3. Comparative pharmacokinetic experiments in plasma and various tissues.

^{14}C-dantrolene sodium, which was a gift from the Norwich Pharmacal. Co. Norwich N.Y., was supplemented with cold dantrolene sodium. Concentrations of 2 mg/kg were administered into the tail-veins of concious rats by bolus injection. The animals were decapitated at different time intervals, blood was collected and the heart and the tibialis anterior muscle were removed and washed for 15 minutes in a cooled Krebs/Hepes solution to remove extracellular label. The tissues were homogenised in three parts of distilled water; 0.5 ml samples were dissolved in 15 ml Plasmasol scintillation fluid and each sample was counted for 10 minutes in a Isocap-5 scintillation counter for total radioactivity.

In a separate series cold dantrolene sodium in the same dose as in the radioactive experiments (2 mg/kg) was administered by injection into the tail-vein and at various time intervals the rats were decapitated. Blood was collected and the concentrations of dantrolene sodium were estimated in plasma, using a modification of the spectrofluorometric method described by Hollifield et al, (1973).

The results were expressed as the mean $\pm$ S.E.M.

3. Results.

3.1. Dose-maximum effect relationship.

Dantrolene sodium gave a dose dependent reduction of the twitch tension of the tibialis anterior muscle of the rat in vivo (fig. 1). The maximal block was achieved within 3 - 5 minutes after bolus injection. After 2 mg/kg the maximal block was 46.9% $\pm$ 1.3%. Recovery to control values of the twitch tension was very slow, both after i.v. and oral administration, the half time of recovery (i.v.) being about 80 minutes.

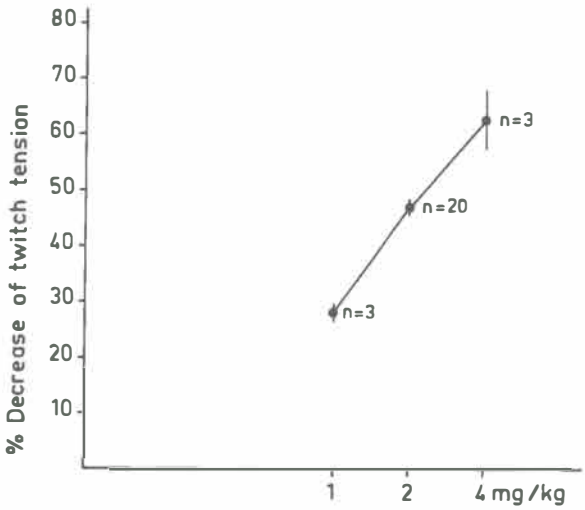

Fig. 1: Dose response curve of dantrolene sodium on the twitch tension of the rat tibialis anterior muscle.
Each point represents mean ± SEM.

3.2. Plasmaconcentration - effect relationship.

On average a maximal block of 47% developed after 2 mg/kg dantrolene sodium intravenously. A mean plasmaconcentration of dantrolene of 5.8 µg/ml was found then.

In table I the plasmaconcentrations and the times at various degrees of block are given. It can be derived from the data (table I, last column) that the ratio between recovery of muscle contractions and the decline in log plasma concentration is not constant in the time. This is visually reflected in fig. 2 which gives the individual plasma-concentrations at four degrees of block versus time.

If a direct relationship between plasmaconcentration and effect were to be present, one might expect that identical degrees of block should involve, with some random variation, approximately identical plasma concentrations. This is not the case however, since the plasmaconcentrations for identical degrees of block are correlated with time ($p < 0.05$ for 60, 75, 90%), thus indicating the existence of a barrier between locus of action and central compartment.

After oral administration of 25 mg/kg dantrolene sodium a maximum twitch

Degree of block at which rats (n) were sacrified	Time after injection (min. ± SEM)	Concentration of dantrolene sodium (µg/ml ± SEM)	% decrease in log plasmaconcentration/% recovery
maximal (n=4)	3.5 ± 0.35	5.8 ± 0.4	
			0.028
60% recovery (n=5)	18.3 ± 2.3	3.7 ± 0.5	
			0.031
75% recovery (n=5)	81.2 ± 16.8	1.2 ± 0.2	
			0.020
90% recovery (n=5)	104.8 ± 16.4	0.6 ± 0.2	

Table 1: Plasmaconcentrations of dantrolene sodium at various degrees of block. Rates of decline of plasmaconcentration and recovery from paralysis are compared in the last column.

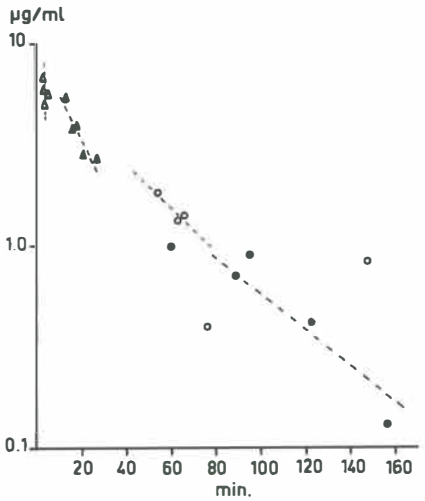

Fig. 2: Plasmaconcentrations corresponding with various degrees of block, against time.

Δ concentrations at maximal decrease of the twitch tension

▲ concentrations at 60% recovery

O concentrations at 76% recovery

● concentrations at 90% recovery

depression of 38% (±1.8%) developed after 14 (±1.7) min (n=5). The corresponding mean plasmaconcentration was 3.6 ± 0.4 µg/ml. Though this corresponds with the i.v. data, different relations between duration of block and plasmaconcentration from those after i.v. injection may be found, since absorption from the G.I. tract is probably still in progress.

3.3. The half life of dantrolene sodium in blood and tissue.

Fig. 3 shows the decay curves of total radioactivity (i.e. dantrolene sodium plus eventual metabolites) in rat plasma, tibialis anterior muscle and heart muscle after i.v. administration. All three curves run parallel; the relevant pharmacokinetic data are given in table 2. The plasmacurve can be described according to a two compartment pharmacokinetic model and the figures suggest that both skeletal muscle and heart muscle belong to the plasma i.e. central compartment. This is in agreement with the rapid onset of action of dantrolene sodium

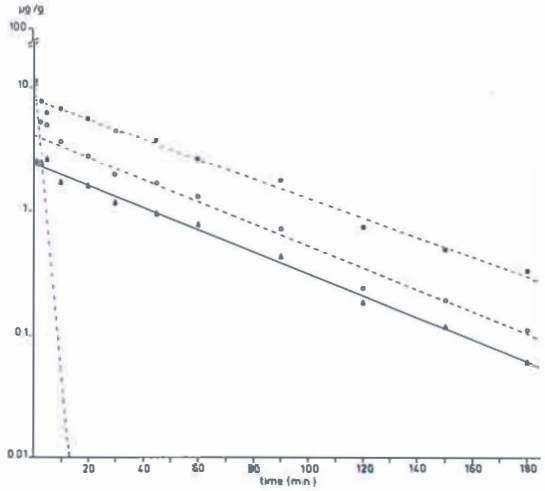

Fig. 3: Concentration/time curve of dantrolene sodium plus metabolites in plasma (-●-), skeletal muscle (-▲-) and heart muscle (-o-) after 2 mg/kg labelled dantrolene sodium i.v. in rats (n=2).

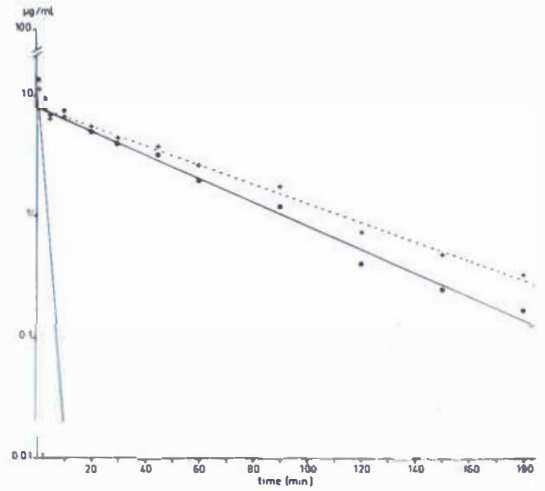

Fig. 4: Concentration/time curve of dantrolene sodium (-+-) and dantrolene sodium plus its metabolites (-●-) in plasma after 2 mg/kg labelled dantrolene sodium i.v. in rats (n=2).

in the tibialis anterior muscle, but not with the slow recovery which suggests that there might be a third, very small compartment, which fills very rapidly, but which has a very slow release. Moreover it can be seen that at any time the concentration in plasma is about four times that in skeletal muscle and only twice the concentration in heart muscle.

In fig. 4 the plasmaconcentration-time curve of dantrolene sodium plus its metabolites, and the plasmaconcentration curve of dantrolene sodium alone is given. The difference between these two curves represents the metabolites, probably for a major part the assumed 5-OH derivate, which is the major active metabolite (Ellis and Wessels, 1978). Since 5-OH dantrolene is not available in labelled form, exact concentrations could not be determined. Both curves run roughly parallel and there is no marked difference between the concentrations. In view of this it is acceptable to use the combined curve in relation to the muscle relaxant effect.

The apparent volume of distribution of dantrolene sodium was estimated with the formula:

$$V_D = \frac{D.\alpha}{A.\beta + B.\alpha}$$

(the parameters A, B, α and β were obtained by iterative peeling).

V_D was relatively small (24 %) probably representing the extracellular water. The maximal block of 47 % decreased by 50 % in about 80 minutes.

Discussion.

The concentration-time relation (fig. 2) and the disproportionality between log plasmaconcentration and recovery in the time (table 2) indicate that the site of action of dantrolene sodium is not in the central i.e. plasma compartment. This is not surprising, since it is reported that the drug acts intracellularly (Ellis and Carpenter, 1974). Since the semi log plasmaconcentrations and muscle decay curves run parallel, this observed discrepancy between plasma concentrations and effect holds also true for the peripheral compartment.

The observed discrepancy may be due to several causes. First, it may be possible that dantrolene sodium does not follow a linear relationship between the log concentration at the receptor site and effect. It is unlikely that during the β-phase in our experiments an overdosage was

		Plasma		skeletal muscle	heart
		dantrolene sodium	dantrolene sodium + metabolites	dantrolene sodium + metabolites	dantrolene sodium + metabolites
A	(µg/ml)	10.9			10.46
α	(min^{-1})	0.618			0.508
B	(µg/ml)	8.0	7.74	2.40	4.15
β	(min^{-1})	0.0225	0.0180	0.0206	0.0207
V_c	(l/kg)	0.105			
V_d	(l/kg)	0.24			
k_{12}	(min^{-1})	0.3153			
k_{12}	(min^{-1})	0.2746			
k_{10}	(min^{-1})	0.0506			
$T_{\frac{1}{2}\alpha}$	(min)	1.12			1.37
$T_{\frac{1}{2}\beta}$	(min)	31	38	34	33.5
kel	($l.min^{-1}.kg^{-1}$)	0.00531			

Table 2: Pharmacokinetic parameters of dantrolene sodium in plasma and dantrolene sodium plus metabolites in plasma, skeletal muscle and heart muscle.

present, since we found a linear dose response curve (fig. 1) in vivo, while concentrations that gave approximately 47 % block were used. Secondly, dantrolene sodium may remain much longer at the receptor site than in the surrounding intracellular, non-reactive space. However, a third compartment representing these receptor sites (i.e. Ca^{++} binding places in the sarcoplasmatic reticulum) is very small in relation to the intracellular space, and thus pharmacokinetic details concerning this "effective" compartment will be completely obscured during the β -phase and will be below detection level later on. Detailed investigations on binding affinity of isolated sarcoplasmatic reticulum might throw more light on this problem. Finally, an active metabolite might interfere and be responsible for the differences between effect and tissue concentration. Even if the major metabolite, 5-hydroxydantrolene, were equally potent as the parent drug, the differences between half lives of metabolite and parent drug are too small to explain this discrepancy. Thus the second explanation, intensive binding to the receptor site is the most likely one. The equilibrium between plasma and biophase is only reached in a later state, which may indicate that the estimation of plasmaconcentrations of dantrolene sodium in relation to effect is only meaningful under steady-state conditions. In rats we found a peak effect after oral administration of a huge dose (25 mg/kg). Plasmaconcentrations were comparable with those after i.v. injection of approximately equipotent doses.

Herman (1972) found peak effects of 40 - 70 % in patients 4 hours after oral ingestion of approx. 2 mg/kg dantrolene sodium, and concomittant plasmaconcentrations of 1.25 µg/ml.

Monster (1973) observed plasmaconcentrations of 0,7 - 1.45 µg/ml, 3 - 6 hours after oral administration of 100 - 125 mg dantrolene sodium (approx. 1.5 mg/kg). Species dependent rate constants of absorption and distribution into muscle and re-entry into the central compartment most probably cause the difference with our experiments.

In conclusion we may say that the pharmacokinetic behaviour of dantrolene sodium in rats offers an explanation for the long-lasting effects of the drug. Though the complex of absorption, distribution, and excretion kinetics in man will probably differ from that in rats, it may be expected that also in man only steady-state plasmaconcentrations will correlate with effect.

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CHAPTER VI

THE RELATIONSHIP BETWEEN PLASMA CONCENTRATIONS AND THE EFFECT OF DANTROLENE SODIUM IN MAN.

1. Introduction.

Dantrolene sodium is a muscle relaxant that has been widely used in the treatment of spasticity. Little is known, however, about its pharmacokinetics in relation to effect, which is especially important for drugs chronically given. In animal studies the pharmacokinetics of dantrolene sodium followed a two compartment model, whereby the recovery from relaxation of the skeletal muscle is much slower than the elimination of dantrolene sodium from plasma (Ch. V).
In man the effect of dantrolene sodium on skeletal muscle and on spasticity in relation to the plasmaconcentrations of dantrolene sodium and its major metabolite i.e. 5-hydroxy dantrolene was investigated.

2. Subjects and methods.

The effect of a single oral gift of dantrolene sodium in relation to plasmaconcentrations was studied: 1. in healthy volunteers by measuring the influence on the twitch tension: 2. in spastic patients on the decrease of muscle hypertonia. Both methods are discussed in detail in the following paragraphs.

2.1. The effect of dantrolene sodium on the twitch tension.

8 Healthy volunteers, 7 males and 1 female, their age ranging from 20 to 28 years, participated in this study. A force-displacement transducer was attached to the right arm of the volunteer, who lay in a supine position. The transducer was firmly secured into the hand by bandaging, while the thumb was free to press the transducer. The whole arm was fixed to a table at the side of the bed. The ulnar nerve was intracutaneously stimulated at the wrist by square wave, supramaximal stimuli of 0.2 msec duration at a rate of 0.2 Hz, every 15 minutes during 2 minutes, delivered by a Grass S44 stimulator and a Grass SIu5 isolation unit. The isometric twitch tension of the indirectly stimulated m. adductor pollicis was recorded. The control twitch tension

was registered for at least 15 minutes. If this remained constant, the volunteer ingested a dantrolene sodium capsule of 100 mg. The twitch tension was registered in all subjects up to 2½ to 5 hours after the ingestion of the capsule; hereafter the twitch measurements were terminated, since the effect of dantrolene sodium is longlasting, and a longer duration of the experiment caused too much discomfort to the volunteers.

8 to 11 bloodsamples were taken during the experiment at fixed intervals preceded by a control sample. Moreover some samples were taken up to 24 hours after ingestion of dantrolene sodium.

Plasmaconcentrations of dantrolene sodium and its major metabolite 5-hydroxy dantrolene at maximal effect and the effect at peak plasma-concentrations were calculated, the effect being definied as the percentage decrease of control twitch contractions. Furthermore the mean muscle relaxant effect was plotted against the logarithm of different (interpolated) concentrations of dantrolene sodium alone, and of dantrolene sodium together with its major metabolite; correlation coefficients were determined. Moreover we determined the median half life of dantrolene sodium and 5-hydroxy dantrolene from the individual half lives. Half lives were determined by calculation of the regressionline through at least three points in the elimination phase using the least-squares method.

2.2. The effect of dantrolene sodium on spasticity.

This study was carried out in 7 patients, 6 males and 1 female, with spasticity of various origins. Some characteristics of these patients are shown in table 1.

The pathologic condition of the patients was considered to be stable. On clinical examination all patients had muscle hypertonia, which was considered to contribute to their functional disability.

After control evaluation the patient took in a random order either a capsule containing 100 mg dantrolene sodium, or a placebo in an identical capsule. Both doctor and patient were unaware of the nature of the capsule i.e. the study was done under double-blind conditions. Evaluation took place 2, 4, and 6 hours after ingestion of the capsule. The decrease in hypertonia was expressed as a percentage of the control values.

Bloodsamples were taken 1, 2, 4, and 6 hours after ingestion. The plasma

was stored deep frozen until determination.
Two days later, at identical hours of the day, the whole procedure was repeated, while the patient who took the placebo capsule in the first experiment, now received dantrolene sodium and vice versa, i.e. a cross-over study was performed.

Patient	Sex	Age	Origin of spasticity	Severity	Concurrent medication
V	M	32	spinal cord lesion	+++	-
B	F	64	spinal cord lesion	++	Acenocoumarol
T	M	71	hemiplegia	+	hydralazin 3x10 alprenolol 2x400 amytryptilin 3x10
D	M	62	hemiplegia	+	methyldopa 3x250 theophyllin 3x200
K	M	68	spinal cord lesion	++	-
Vr	M	28	spinal cord lesion	+++	-
M	M	48	spinal cord lesion	++	baclofen 3x30

Table 1: Personal data of the patients in the double-blind cross-over trial with dantrolene sodium 100 mg orally versus placebo.

The effect of the drug was defined as the difference between recordings obtained after 100 mg dantrolene sodium capsule and after placebo respectively. The effect was evaluated by quantitative measurement of the muscle hypertonia (see below). Moreover simple cardiovascular parameters i.e. bloodpressure, heartrate and central venous pressure were determined.
The relationship between plasmaconcentrations and effect was examined

in the same way, as it was done in the volunteer study.

2.3. Quantitative measurements.

The force, required from the examiner to move passively the representative lower limb of the patient in the kneejoint, was measured using the socalled RED apparatus. The patient was positioned supine on a bed in a relaxed position with his lower legs hanging down over the edge of the bed. The conditions of the test and the method of registration are described by Kwee (1976). Evaluation of this quantitative measurement is described in Ch. I.

2.4. Determination of dantrolene sodium and 5-hydroxy dantrolene.

The plasmaconcentrations of dantrolene sodium and the 5-hydroxy metabolite were determined spectrofluorometrically using a modification of the method of Hollifield et al, (1973).

The method of Hollifield is based on a combination of solvent extraction, chromatography, and fluorescence characteristics. Since the fluoriscence characteristics of dantrolene sodium and 5-hydroxy dantrolene are alike, chromatography is necessary to separate both compounds. According to the method of Hollifield, the concentrations of 5-hydroxy dantrolene are estimated by calculating the difference between the procedure without column chromatography (direct: dantrolene sodium together with 5-hydroxy dantrolene) and with chromatography (indirect: eluation of 5-hydroxy dantrolene with NaOH, dantrolene sodium extracted with dimethylformamide (DMF).

We obtained good results with the determination of 5-hydroxy dantrolene in the NaOH fraction which passed the Sephadex column, before dantrolene sodium is eluated with DMF. This fraction is discarded by Hollifield. We even found that the total amount of fluorescence of the NaOH fraction (with 5-hydroxy dantrolene) plus the DMF fraction (with dantrolene sodium) was considerably larger than the total fluorescence by direct measurement (5hydroxy dantrolene + dantrolene sodium). In our opinion this indicates that chromatography in some way alters the fluorescence of both dantrolene sodium and the metabolite, since we could confirm by using radioactive dantrolene sodium that the separation procedure is correct. Some other differences with the method of Hollifield:

1. After the Sephadex column we acidify the 4 ml DMF eluate with 1.5 ml 0.3 N HCl to achieve the pH 1-2 which is necessary for a good recovery from this fraction, instead of the 1 ml 0.3 N HCl described by Hollifield.

2. Instead of nitropropane-heptane we use a mixture of pentanone-heptane since this is much less toxic.
3. We set the spectrophotofluorometer with an external standard of 0.1 µg quininesulfate/1 ml 0.1 N H_2SO_4, instead of a 4 µg/ml aqueous dantrolene internal standard, using an Aminco Bowman spectrofluorometer.

3. Results.

3.1. The effect of dantrolene sodium on the twitch tension.

In table 2 the maximal muscle relaxant effect and the maximum plasma concentrations of dantrolene sodium and 5-hydroxy dantrolene, with corresponding times after ingestion of the capsule, are presented.
No decrease of the twitch tension was observed during control experiments.
Following dantrolene sodium 100 mg orally the mean maximum twitch depression was 49.1% ± 9.4% (± S.D.). This effect was reached between 1.15 hours and 3.45 hours after ingestion. The muscle relaxant effect is visualised in fig. 1 and is in accordance with the results obtained by Herman (1972).

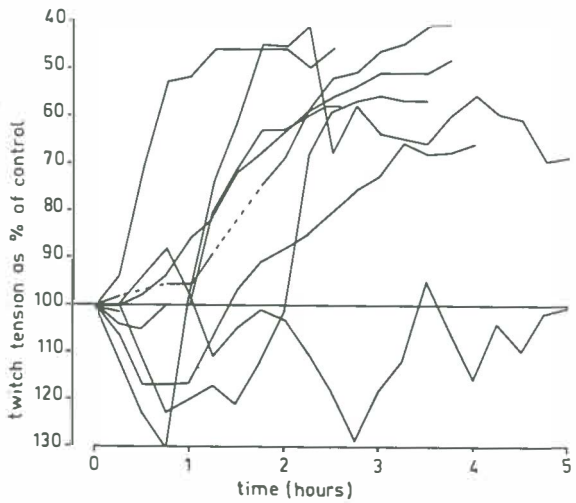

Fig. 1: Individual decrease of the twitch tension plotted against time after a single dose of 100 mg dantrolene sodium orally in 8 normal subjects.

	Sex	Age	max. eff. % control	time/ hours	max. DS µg/ml	time/ hours	max. 5OHD µg/ml	time/ hours	T½DS hours	T½5OHD hours
P.G.	M	20	34	3.15	1.09	2.00	0.53	22.15	22.04	?
F.O.	M	28	-	-	1.14	12.00	0.40	12.00	8.48	20.27
T.W.	M	24	54	1.15	1.72	1.00	0.34	2.05	9.07	29.44
B.C.	M	25	42	2.30	1.34	8.25	0.45	8.25	6.38	15.54
D.W.	M	25	44	3.00	0.69	4.00	0.33	6.45	4.53	11.33
H.P.	M	25	59	2.15	1.40	1.30	0.20	1.30	5.25	?
A.F.	F	24	59	3.30	1.51	4.00	0.39	7.40	5.35	15.45
J.T.	M	24	52	3.45	1.06	4.00	0.52	4.00	4.10	8.10
	mean:		49.1	2.47	1.24		0.39			
			± 9.4	± .52	±0.32		±0.11			

Table 2: Maximum decrease of the twitch tension after ingestion of 100 mg dantrolene sodium expressed as a percentage of control values with corresponding times, the maximum plasmaconcentrations of dantrolene sodium (DS) and 5-hydroxy dantrolene (5OHD).
In the last 2 columns the indivudual half lives are given.

One volunteer (F.O.) did not exhibit depression of the twitch tension within 5 hours after the start of the experiment and therefore a concentration-effect relationship could not be established in this individual. Yet he experienced a moderate but unmistakeble effect (drowsiness and weakness in the legs) 12 hours after ingestion of the capsule. A mean maximum plasma concentration of 1.24 ± 0.32 µg/ml (± S.D.) was obtained. The times, at which the maximum plasmaconcentrations of dantrolene sodium were reached, varied considerably, namely between 1 hour and 12 hours. The mean maximum plasmaconcentration of 5-hydroxy dantrolene was 0.39 + 0.11 µg/ml (± S.D.); the individual peak concentrations were reached from 1.30 hours to 22.15 hours after ingestion. The individual plasma concentrations of dantrolene sodium are shown in fig. 2 and those of 5-hydroxy dantrolene in fig. 3. The individual half lives also showed much variation. The median half life of dantrolene sodium was 6.06 hours, ranging from 4.10 hours to 22.15 hours. For 5-hydroxy dantrolene a median half life of 15.50 hours was calculated, ranging from 8.10 hours to 29.44 hours. There was a correlation between the mean muscle relaxant effect and corresponding log plasma concentrations, as is shown in fig. 4. This was found for dantrolene sodium alone ($p<0.001$), but also for dantrolene sodium together with 5-hydroxy dantrolene ($p<0.001$).

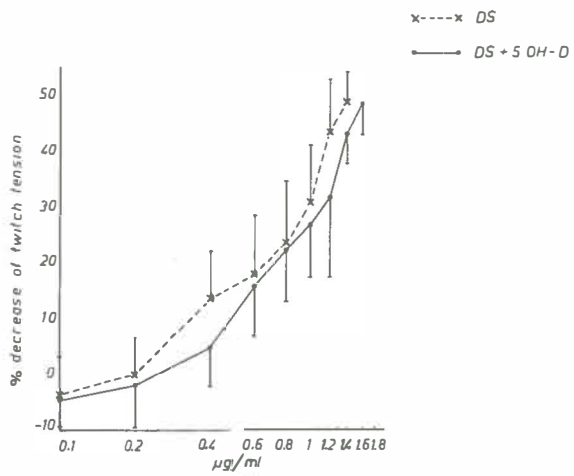

Fig. 4: Log concentrations of dantrolene sodium (x--x) and dantrolene sodium together with 5-hydroxy dantrolene (●-●) with corresponding percentage decrease of the twitch tension (± SEM).

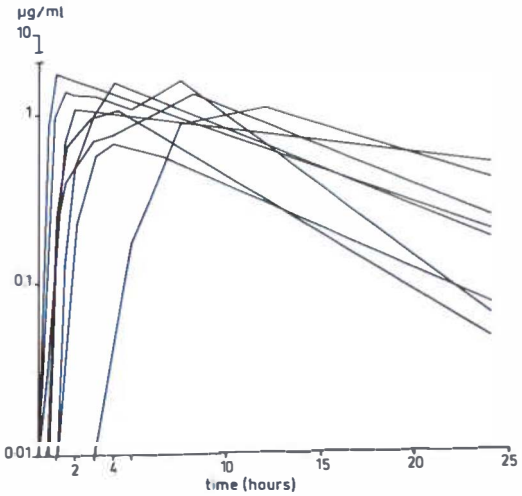

Fig. 2: Individual plasmaconcentrations of dantrolene sodium logarithmically plotted against time after a single dose of 100 mg dantrolene sodium orally in 8 normal subjects.

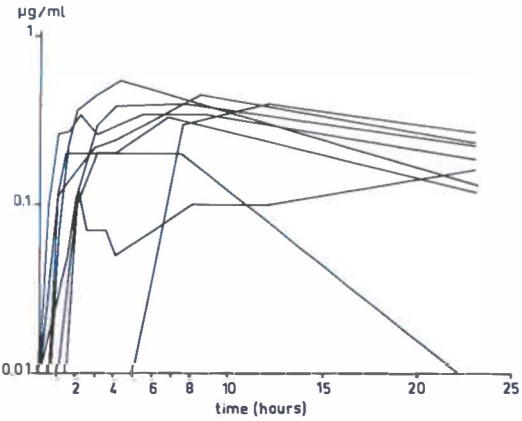

Fig. 3: Individual plasmaconcentrations of 5-hydroxy dantrolene logarithmically plotted against time after a single dose of 100 mg dantrolene sodium in 8 normal subjects.

We could not establish a correlation between maximum plasmaconcentrations of dantrolene sodium and corresponding effect. But if the concentrations of dantrolene sodium and of 5-hydroxy dantrolene were taken together, a significant correlation with the maximum twitch depression was observed ($p<0.05$). In three volunteers the plasma concentrations of dantrolene sodium decreased, while the effect still increased; the opposite did not occur. This seems to be in accordance with our findings in rats (Ch. V), where a considerably longer duration of effect was found compared with plasmaconcentrations.

All volunteers showed marked sedation and dizziness at the time of the maximum plasma concentrations of dantrolene sodium.

3.2. The effect of dantrolene sodium on spasticity.

In this double-blind cross-over study 6 out of 7 patients exhibited a marked decrease of spasticity after a single dose of 100 mg dantrolene sodium orally taken.

Patient	max. effect % control	time/ hour	max. conc. of DS µg/ml	time/ hour
V	51	2	1.75	2
B	40	6	0.82	6
T	25	4	0.95	4
D	50	2	1.86	2
K	76	6	1.40	6
Vr	6	6	0.96	6
M	68	4	0.80	6

Table 3: Maximal decrease of spasticity after ingestion of 100 mg dantrolene sodium (expressed as a percentage of the spasticity under placebo treatment) with corresponding times, and the maximum plasmaconcentrations of dantrolene sodium with corresponding times.

As can be seen in table 3, the effect of dantrolene sodium was reached between 2 and 6 hours after ingestion of the capsule. In 6 patients the maximum effect and the peak plasma concentration of dantrolene sodium were reached at the same time. In 5 out of 7 patients the plasma levels followed the effect quite reasonably. But, due to enormous variability between the conditions of the patients, no correlation could be found between mean plasma concentrations and effect. Nevertheless, apart from one patient with severe and variable spasticity, it was found in all patients that plasma concentrations higher than 0.3 µg/ml were associated with a superior effect of dantrolene sodium compared with placebo.
Like the volunteers, nearly all patients noticed marked sedation and dizziness at the time of the peak concentration of dantrolene sodium. In two cases there was a marked increase in systolic and diastolic bloodpressure compared with placebo values. Patient T. showed an increase from 165/100, the highest bloodpressure measured during placebo, to 190/125 during dantrolene sodium treatment, patient Vr. from 140/90 to 180/100.

4. Discussion.

We established a correlation between the plasma concentrations of dantrolene sodium and its effect on the twitch tension. If the plasma concentrations of 5-hydroxy dantrolene were added, again a correlation with effect was observed (fig. 4).
Despite of these findings, it is not possible to give a reliable impression of the potency ratio between dantrolene sodium and 5-hydroxy dantrolene. Though fig. 4 shows a correlation between log concentration and effect, it cannot be accepted that this relationship is linear, because of the "tight receptor binding" phenomenon. In addition there are suggestions (Ellis and Wessels, 1978) that the dose response curve of dantrolene sodium and 5-hydroxy dantrolene do not run parallel.
Supposed that 5-hydroxy dantrolene is also active in man, this metabolite with its much longer half life (15.50 hours) might bring about that the effect is still increasing, while the plasma concentrations of dantrolene sodium are decreasing. Furthermore it is likely that "tight receptor binding", as seen in animals (Ch. V), contributes to the long-lasting muscle relaxation.

In comparison with the animal experiments (Ch. V) it is obvious that man is more sensitive to dantrolene sodium. A comparable muscle relaxant effect was obtained in rats with 2 mg/kg i.v. and in man with about 2 mg/kg orally, while the plasma concentrations in rats were 4.5 times higher.

In our study we could not establish a correlation between plasma concentrations of dantrolene sodium itself or in conjunction with its major metabolite, and the effect on spasticity. This may be due to a large interindividual spread in responsiveness, but more likely it is caused by the variability of the spasticity symptomatology. The symptoms may change considerably from day to day, but also during the day. These factors are the cause that quantative measurement of improvement is very difficult.

The effect of dantrolene sodium on the twitch tension and on spasticity can be explained by the action on the extrafusal fibers of the muscle. The extrafusal fibers are the target organ i.e. the effector component of the reflexes that are abnormally excitated in spasticity. Moreover the effect of dantrolene sodium can be attributed to the action on the intrafusal fibers of the muscle spindles. The spindle is a part of the gamma loop, which modulates the tone of the muscle. In cats the drug prevents the increase in spindle discharge following the stimulation of fusimotor fibers (Zorchyta et al, 1971). Thus the spindle excitates the disinhibited spinal reflexes in a lesser way.

The depression of the isometric twitch tension caused by dantrolene sodium is more pronounced than the influence on the tension after tetanus stimulation (Heald and Matsumoto, 1972; Monster et al, 1974; Migletta, 1977). At at the end of our twitch experiments, at the moment of maximal depression (49%), we found that the volunteers could walk normally, but only noticed that they had "heavy legs".

The effect of one single dose of 100 mg dantrolene sodium on spasticity may partly be due to central effects. Sedation, which was noticed in nearly all cases, could have affected spasticity. This central effect is absent or only transient, if dantrolene sodium medication is adjusted slowly.

In conclusion: we found a very considerable decrease of twitch tension in normal subjects after 100 mg dantrolene sodium. This could not be observed in patients who suffered from spasticity. In the latter group

a concentration-response relationship appeared methodologically impossible inherent to the individual variability of spasticity. Yet clinical observations showed that concentrations higher than 0.3 µg/ml did consistently better than placebo in our patients. It remains to be seen, whether these concentrations will be achieved after long-term administration. These questions are discussed in the following chapter, where results of a long-term open trial with dantrolene sodium, related to plasmaconcentrations, toxicity and effect, are reported.

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CHAPTER VII

THE EFFECT OF DANTROLENE SODIUM IN RELATION TO BLOOD LEVELS IN SPASTIC PATIENTS AFTER PROLONGED ADMINISTRATION.

The purpose of the present study was not primarily to quantify therapeutic drug effects. Of the patients available for this study most had been treated with various drugs and other therapies. Dantrolene sodium was given in increasing doses to these patients and eventual changes were noted; the main accent of this study was focused, however, on collection of pharmacokinetic and toxicity data.

1. Patients and methods.

27 In-patients (23 males and 4 females) with spasticity of various origin were added to the study; 20 patients were admitted to the rehabilitation hospital "De Hoogstraat" at Leersum and 7 patients to the rehabilitation hospital "Heliomare" at Wijk aan Zee. Their ages ranged from 19 to 68 years with a mean of 40.5 years. The spasticity, which was supposed to be stable, was in 22 cases of spinal origin and in 5 cases of cerebral origin (table 1). Patients with cardiovascular, metabolic, and present or past liver diseases were excluded from the study.

	Number of patients
SPINAL CORD PATHOLOGY	
Quadriplegia	9
Paraplegia	10
MULTIPLE SCLEROSIS	1
SUPRASPINAL PATHOLOGY	
Hemiplegia	2
Cerebral palsy	1
Contusio cerebri	2

Table 1. Diagnosis in 25 patients. 2 Patients with spinal cord pathology did not start, because of slight raised liver functions.

The following complaints were outstanding: spasticity in 16 patients, disturbance of activity of daily living (ADL) in 5 patients, nursing difficulties in 2, disturbance of motor performance in 4, pain in 4 patients and clonus in 1 patient.

Dosage.

Dantrolene sodium (in capsules of 25 and 100 mg), which was a gift of Norwich Benelux B.V., was supplied according to the following dosage schedule:

25 mg b.i.d.
25 mg q.i.d.
50 mg q.i.d.
100 mg q.i.d.

until the "optimal" dose of the drug was achieved, provided the maximum daily dose of 400 mg was not exceeded.

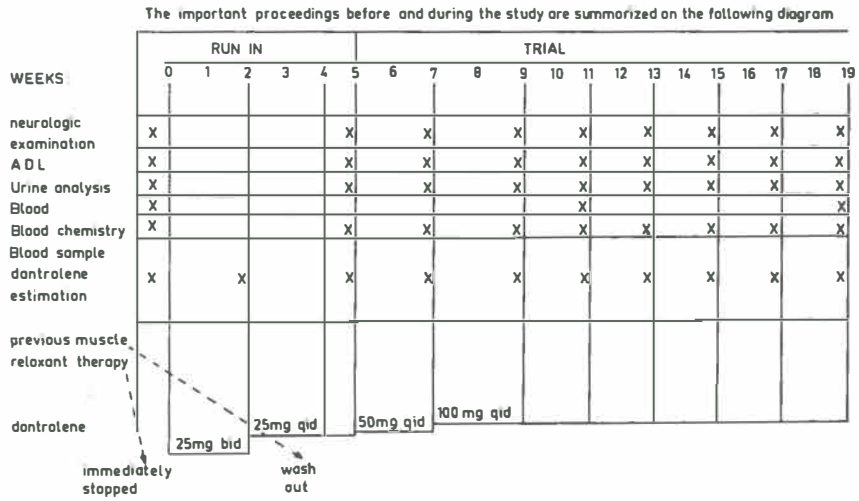
As "optimal" the daily dose was considered with which the therapeutic goal was obtained without inacceptable side effects. The speed of dosage titration depended on the individual patient's tolerance of the drug. Patients were kept on a particular dose level for 2 weeks before the next higher dose was given. Each patient maintained his "optimal" dose till the end of the study. Patients who displayed drug intolerance were dropped from the study. In patients with intercurrent illnesses, during which dantrolene sodium therapy seemed to be inappropriate, the drug was temporarily discontinued, but it was resumed after recovery.

Base line and follow up measurements.

Data were collected before the start of the study, and control measurements were made before and at 14 days intervals during the study. They consisted of:

1. Complete neurologic examination during which resistance against passive stretch (according to the Ashworth rating scale) was measured and furthermore strength, clonus and tendon jerk reflexes (according to predetermined rating scales).
2. Activity of daily living (ADL) evaluation.
3. Laboratory observations including checks of haemoglobin, haematrocrite, sedimentation rate, white cells, platelets, as well as determination in serum of ureum, creatinine, direct and indirect bilirubin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase. The urine was analysed on red and white

bloodcells, casts, protein and sugar. The same follow up measurements were done before and during the trial at two weeks intervals. The diagram shows the pertinent proceedings.



A 5 ml blood sample was collected every 2 weeks approximately 3 hours after the morning ingestion for the estimation of drug levels. Though we aimed at collecting these samples at constant intervals after ingestion of the morning dosis, due to hospital routine it could not be prevented that some variations occurred. The samples (cells + plasma) were deep-frozen until analysis, performed in the United States by the Eaton Laboratories, Norwich, N.Y. Dantrolene sodium, 5-hydroxy dantrolene and the acetylated metabolite were measured according to the method of Hollifield and Conklin (1973).
At the end of the 19 weeks study the "overall clinical result" was evaluated. This stands for any meaningful change in spasticity in all its aspects, as judged by the doctor and scored as improvement, equal and worse. The various blood levels after prolonged administration of dantrolene sodium were compared at different daily dose levels. Changes (in a set of consistent trends) in the spastic conditions and side effects were compared with the dose levels and the blood levels of dantrolene sodium.
If before the trial the patient was using other muscle relaxants (diazepam or baclofen), this medication was stopped (if possible). Depending on the condition of the patient this medication was either withdrawn immediately or washed-out during the first 3 weeks (see diagram).

2. Results.

Because of slightly raised liver functions, 2 quadriplegic patients were excluded from participation. 17 Of the 25 patients terminated the 19 weeks study, while 8 patients had to stop earlier; 3 patients because of non-drug related reasons (operation, removal or lack of motivation), 5 patients were withdrawn, because of intolerable side effects (incontinence: 1; muscle weakness: 2; pain: 2). The minimum duration of participation was 5 weeks. The daily dose of the patients with intolerable side effects never exceeded 100 mg per day.

The following data are based on the analysis of 25 patients. In table 2 the data of the neurological examinations are summarized.

	passive* resistance	paresis	reflex hyperactivity	clonus*	involuntary* movements	pain*	ADL
improvement	12	7	20	16	3	5	13
no change	8	16	4	1	6	5	7
deterioration	3	2	1	3	5	7	3

Table 2. Results of dantrolene sodium treatment on various parameters of spasticity in 25 patients.
*These symptoms were not present in all patients.

Dantrolene sodium gave an improvement of the resistance against passive movement in 12 of the 23 patients with increased resistance. (Two other patients had no muscle spasms in their limbs, but in the abdominal muscles, which made proper evaluation impossible). Most patients exhibited a decrease of reflex activity and clonus. In contrast with the number of patients with improvement of involuntary movements and pain during dantrolene sodium medication, more patients complained of deterioration of

involuntary movements and pain.
In 7 paretic patients muscle strength improved during the study, while 13 patients showed a more or less distinct improvement of ADL functions; deterioration of these functions was observed in 3 patients. It should be noticed that the regular rehabilitation program was continued for each patient during the study. So, it is difficult, especially as far as muscle strength and ADL functions are concerned, to discriminate between the drug effect and the effect of the other therapeutic measures.
Before the start of the study, 11 patients were on a different muscle relaxant (baclofen: 4; diazepam: 4; baclofen combined with diazepam: 3). This medication was washed-out during the first 3 weeks in 10 patients, while one patient remained on concurrent diazepam medication during the whole study.
Table 3 lists the various dose levels that were obtained and the number of corresponding patients. The mean blood levels of dantrolene sodium and

Daily dose level mg	Nr. patients	Mean DS conc. ± SD (n) (µg/ml)	Mean 5OHD conc. ± SD (n) (µg/ml)
50	-	0.37 ± 0.29 (25)	0.08 ± 0.08 (25)
100	8	0.59 ± 0.21 (25)	0.17 ± 0.11 (25)
150	1		
200	6	1.08 ± 0.55 (16)	0.44 ± 0.30 (16)
400	10	1.44 ± 0.89 (10)	0.51 ± 0.36 (10)

Table 3. The mean blood level of dantrolene sodium (DS) and 5-hydroxy-dantrolene (5OHD) determined from data of all patients (n) at each dose level. Column 2 indicates the number of patients that remained on the different daily dose levels.

5-hydroxydantrolene were determined at each dose level after 14 days of administration. If in those patients, who gradually increased their dose till 400 mg per day, the daily doses are plotted against the blood levels of dantrolene sodium and those of 5-hydroxydantrolene, a linear dose-

concentration relationship is found with significant differences between the corresponding blood levels for the 50, 100 and 200 mg daily dose levels respectively, but not between the 200 and 400 mg dose levels. These results are shown in fig. 1.

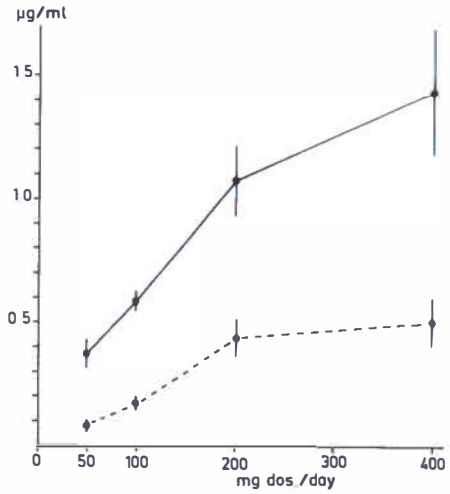


Fig. 1. Mean blood levels (± S.E.M.) of dantrolene sodium (—) and 5-hydroxydantrolene (--) at different daily doses.

The ratio between dantrolene sodium and 5-hydroxydantrolene did not change significantly in the course of weeks. After prolonged administration of both 100 and 400 mg daily, blood levels remained fairly constant; approximately 0.7 µg/ml in the patients who remained on the 100 mg dose, but only twice that concentration (approximately 1.4 µg/ml) in the 400 mg group. The acetylated metabolite was only occasionally detectable; its levels were always below the detection level (approximately 0.8 µg/ml).

The "overall clinical result" of dantrolene sodium therapy, as judged by the doctor in charge of the patient and the patient himself, is shown in table 4 in relation to the mean blood levels of dantrolene sodium and 5-hydroxydantrolene. No correlation was found between blood level and

Results	Nr. patients	Mean DS conc. ± SD (µg/ml)	Mean 5OHD conc, ± SD (µg/ml)
Marked	3	1.19 ± 0.57	0.44 ± 0.30
Moderate	4	0.66 ± 0.22	0.16 ± 0.04
Slight/ Insufficient	7	1.17 ± 0.54	0.46 ± 0.24
Absent	11	1.09 ± 0.57	0.43 ± 0.27

Table 4: The "overall clinical result" which was observed in the number of patients with matching mean bloodlevels (± SD) of dantrolene sodium (DS) and 5-hydroxydantrolene (5OHD).

effect, which indicates that the effect on spasticity after prolonged administration of dantrolene sodium may occur at individually very different blood levels. These results could be in accordance with those of Ch. VI, which showed no correlation between plasmaconcentrations of dantrolene sodium (alone or together with 5-hydroxydantrolene) and the muscle relaxant effect in spastic patients after a single dose of 100 mg either. It should be noticed, however, that the mean blood levels in the group of patients with a moderate effect of dantrolene sodium therapy, are much lower than those in the other 3 groups. If the daily dose levels between the group with a positive result (marked + moderate) and the group with a negative result (slight/insufficient + absent) are compared (table 5), it is seen that 5 out of 7 patients in the first group remained on 100 mg per day, whereas only one patient received 400 mg per day. In the second group, however, only 3 out of 17 patients remained on 100 mg, while 9 patients received the maximum dose of 400 mg per day.
It appears that the results in those patients who received more than 100 mg, were significantly worse than in those who remained on a 100 mg daily dose (Fisher exact probability test, $p \leq 0.01$). One may also say that the majority of patients did not react favourably, even at the highest dose

Daily dose level (mg)	Positive nr. patients	Result negative nr. patients
50	-	-
100	5	3
150	-	1
200	1	5
400	1	9

Table 5. The maximum daily dose level of dantrolene sodium and the number of patients with a positive or negative result from medication.

levels.

4 Patients (out of 7), who experienced a beneficial effect from the medication, continued with dantrolene sodium after the study. The other 3 withdrew; 2 patients because of disturbing side effects (skin rash, muscle weakness and pain) and 1 patient, because of disappearance of spasticity.

Table 6 lists the side effects that occurred during the study. The total number of side effects exceeded the number of patients. In most cases the side effects were not transient, since they were observed twice after 2 weeks intervals of the evaluation. The mean blood levels of dantrolene sodium and 5-hydroxydantrolene at which certain side effects occurred, were also determined. Table 6 shows the different mean blood levels and daily dose levels for those side effects that were observed in more than two patients. Though side effects were noticed after different dosages, the concomittant blood levels, at which they occurred, did not differ (apart from those patients with drowsiness and muscle weakness) from the values found in patients without side effects. It can be seen that in those patients who experienced muscle weakness, this started at relatively low daily dosage and blood levels. Anorexia was frequently seen, but it only occurred at a relatively high daily dosage.

Examinations of the bloodchemistry and liverfunctions revealed no

Side effects	Nr. observations	Mean daily dose level ± SD (mg)	Mean conc. DS ± SD (µg/ml)	Mean conc. 5OHD ± SD (µg/ml)
Dry mouth	2			
Anorexia	7	300 ± 129	1.26 ± 0.52	0.42 ± 0.23
Nausea	4	233 ± 153	1.10 ± 0.59	0.59 ± 0.32
Pyrosis	1	-	-	-
Incontinence	2	-	-	-
Obstipation	1	-	-	-
Impotency	1	-	-	-
Drowsiness	6	216 ± 147	0.62 ± 0.56	0.33 ± 0.27
Depression	3	233 ± 153	1.22 ± 0.70	0.53 ± 0.29
Muscle weakness	4	112 ± 25	0.65 ± 0.54	0.17 ± 0.09
Skin rash	1	-	-	-

Table 6. Total amount of cases showing side effects. (the number of side effects exceeded the total number of patients.) Mean dose level of dantrolene sodium and mean blood-levels of dantrolene sodium (DS) and 5-hydroxydantrolene (5OHD) ± SD are given for those side effects that were registered more than twice.

abnormalities. Neither deviation of the normal mean values normal mean values nor rises to pathological values were observed in any individual.

3. Discussion.

In this open study lasting 19 weeks a muscle relaxant effect of dantrolene sodium, which was to be expected beneficial to the spastic patients, could only be established in 7 out of 25 patients. The effect on neurological signs, for example resistance against passive movements and reflex activity, was much more favourable. This implies that these parameters do not give a good reflection of the "overall clinical result". This was also reported in other studies (see introduction Ch. III).

It was remarkable that those patients who had a moderate or good effect, were mainly part of the group of patients who remained on 100 mg dantrolene sodium per day. In contrast, all (except one) patients who achieved 400 mg per day, had insufficient or no beneficial effect from dantrolene sodium therapy. The reason that patients who recieved 400 mg daily doses did worse than patients who remained on 100 mg, maybe that the first category suffered from a type of dantrolene sodium resitant spasticity that will not react on any dose. Furthermore, the relatively low blood levels after high doses may partly offer an explanation. It should also be noticed that the blood levels of dantrolene sodium after 400 mg per day chronically did not differ significantly from plasmalevels after 100 mg single dose reported in Ch. VI (1.44 ± 0.89 µg/ml versus 1.24 ± 0.32 µg/ml). There are several possibilities causing this phenomenon. Induction of the metabolising enzymes in the liver is not a likely explanation, since the ratio between dantrolene sodium and metabolites did not show a significant change after prolonged administration. A small decrease of this ratio between the 2nd and 5th week is probably due to the difference in half lives (see Ch. VI). An increase of rate of metabolism can not explain the lower blood levels, because then this should not only be observed in the highest dose range. If the metabolism of dantrolene sodium follows Michaels-Menten kinetics, as may be assumed, the velocity of the process will increase with increasing substrate concentrations till the metabolising enzyme is saturated; thereafter the opposite phenomenon will occur, resulting in a non-linear increase of plasmaconcentrations. Thus, a different cause, not related to changes

in metabolism, is more likely i.e. capacity limited absorption.
One of the aims of the present investigation was to study any relationship between dose and bloodlevel on one hand and frequency, duration and severity of changes in liver functions on the other hand. To this part of the study we were prompted by several reports of hepatic injuries, including fatal ones (Ogburn et al, 1978; Schneider and Mitchell, 1976; Goodman et al, 1977; Lundin, 1977), that were possibly caused by dantrolene sodium. In several other studies changes in liver functions were reported (Mayer et al, 1973; Chipman et al, 1974; Chyatte and Basmayan, 1973; Chyatte and Birdsong, 1973; Gelenberg and Poskanzer, 1973; Knutsson and Martenson, 1976). Utili (1977) suggested that this complication is only restricted to the higher dose level group. During 19 weeks of treatment we did not observe any abnormality of the liver functions nor even values that tended to rise. This may be due to the small number of patients participating in the study, but also it indicates that, if this phenomenon occurs, its incidence is not very high.
Many side effects were found in this study. The total number even exceeded the number of patients participating in the investigation. Especially anorexia and nausea, muscle weakness and drowsiness were registered. These were not transient, except in a few cases. Especially muscle weakness in 2 patients was disturbing enough to withdraw them from the medication. Drowsiness and muscle weakness were also reported by others to be major side effects (Gelenberg and Poskanzer, 1973; Lietman et al, 1974; Jonsson et al, 1975; Knutsson and Martenson, 1976; Schmidt et al, 1976; Joynt, 1976; Levine et al, 1977). Schmidt et al (1976), who treated 42 patients with spasticity due to multiple sclerosis with maximum dose levels of 300 mg per day, found these side effects, also at low dose levels in contrast with diazepam. We found that muscle weakness and drowsiness appeared at lower blood levels of dantrolene sodium than the other side effects, for instance anorexia.
Patients who are easily sedated or patients with borderline strength, may be especially susceptible to these two side effects. As far as the other side effects are concerned, it is suggestive that depression occuring in 3 patients was drug related, since it disappeared after discontinuation of dantrolene sodium. This also happened to the patient who experienced a skin rash, which was also reported by Joynt (1976) in two patients.

In conclusion we may say, that from our study emerges, that under uncontrolled conditions dantrolene sodium is a muscle relaxant with a weak to moderate effect in patients with spasticity; both absorption and effect at doses higher than 200 mg daily are probably poor; many side effects were registered, but we could not find any influence on the liver functions.

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CHAPTER VIII

GENERAL DISCUSSION.

Recent investigations have contributed to the profile of dantrolene sodium. In this chapter the findings of these investigations will be summarized together with the results of our own contributions.

As to additional indications, it should be noted that dantrolene sodium is effective against provocated malignant hyperpyrexia in swine (Harrison, 1975; Jardon et al, 1976; Anderson and Jones, 1976).

As far as the determination of the mode of action is concerned, Van Winkle (1976) observed that in isolated sarcoplasmatic reticulum dantrolene sodium has only affected the release phase of the Ca^{++} movements, since the total amount of Ca^{++} released, was much lower than the total amount of Ca^{++} found in control preparations.

Desmedt and Hainaut (1977) showed that the reduction of the calcium efflux caused by dantrolene sodium resulted from a shift in balance between the Ca^{++} movements into and out of the intracellular storage sites of the sarcoplasmatic reticulum.

It appeared that dantrolene sodium also affects other substrates than the calcium storage sites of the sarcoplasmatic reticulum in striated muscle, because it depressed markedly and irreversibly the frequency of spontaneous minitiature endplate potentials of the frog neuromuscular junctions (Skirboll and Dretchen, 1975; Statham and Duncan; 1976). The authors suggested that dantrolene sodium acts at intracellular Ca^{++} stores other than the mitochondria in the presynaptic terminals. In Chapter IV experiments are described, showing that dantrolene sodium produced a long-lasting dose-dependent reduction of the contractility of the isolated rat heart up to 75% of control values; but also showing that the rat heart appeared to be less sensitive to low concentrations of dantrolene sodium than the skeletal muscle in-vitro, i.e. the dose-response curve was also flatter. This effect on the heart was confirmed by the experiments of Bowman and Khan (1977) on isolated guinea-pig atria; the effect was more pronounced in paired atria than in left (i.e. electrically driven) atria alone. On the other hand recent investigations of Gollan et al (1977) showed no effect of dantrolene sodium on the rat heart in-vitro when using

the Langendorff preparation. It is difficult to give a satisfactory explanation, but it may probably be due to differences between the experimental settings. Gollan (1978) perfused the rat hearts only for 10 minutes with dantrolene sodium; a stable control period lasting for at least 20 minutes could not be obtained; epinephrine was not added to the perfusion fluid. These findings, coupled with the results of recent investigations, which show that dantrolene sodium also depresses the contractility of smooth muscles (Bowman and Khan, 1977; Graves et al, 1978), suggest that the effects of dantrolene sodium are not as specific as was thought initially. Although these data can not be extrapolated to human situations without strong reserve, it might be important to realize that in patients who will possibly be treated with acute, excessive doses of dantrolene sodium, as in malignant hyperpyrexia, unexpected complications may appear in other organs than just the skeletal muscle.

Another field of investigation concerns the development of antagonists of dantrolene sodium. Bowman et al (1977) showed that 4-aminopyridine and quinine are antagonists of dantrolene sodium. We have also observed this property of 4-aminopyridine in rats on the twitch tension.

In Chapter V we have demonstrated that the kinetics of dantrolene sodium in rats can be described according to a two compartment model. In rats there is a discrepancy between the duration of the effect and the plasma-concentrations of dantrolene sodium. A very long half time (10 hrs) of the effect was also found by Kotsias and Muchnick (1978), while a complete recovery from muscle relaxation occurred after 24 hours.

These findings indicate that the receptor of dantrolene is not located in the central compartment. Moreover, our experiments have proved that after i.v. injection of 2 mg/kg dantrolene sodium a moderate muscle relaxation was achieved which was associated with plasmaconcentrations of 5.8 µg/ml. In these experiments the twitch tension decreased by 47% approximately. In-vitro 50% decrease of heart contractility was reached at 8 µg/ml. This indicates that concentrations, at which the effect of dantrolene sodium on the rat heart can be observed, may be obtained in-vivo. It should be mentioned, however, that kinetics may influence the effects. In-vitro the rat heart was perfused with dantrolene sodium for half an hour, while in vivo peak concentrations were only present for some minutes. Moreover, protein binding in plasma may cause lower free concentrations. Vallner et

al (1976) demonstrated that binding to albumin does occur, but these authors did not mention to which extent it occurred.

The effect of dantrolene sodium in man has been studied in Chapter VI. After oral administration of 100 mg a mean depression of the twitch tension of 49% was observed at a mean plasmaconcentration of 1.24 µg/ml dantrolene sodium. There was a correlation between the effect and the plasmaconcentration. Man appeared to be more sensitive to dantrolene sodium than rats, because comparable effects were achieved, while the mean plasmaconcentration of dantrolene sodium in rats was much higher than in man (5.8 µg/ml versus 1.24 µg/ml).

A correlation between plasmaconcentrations of dantrolene sodium and effect on spasticity in patients could not be established, though a definite effect in 6 out of 7 patients has been observed after a single dose of 100 mg taken orally. An explanation may be caused by the conditions of the experiment. Due to the slow and erratical absorption and the long half life of dantrolene sodium, it was necessary to prolong the experiments for quite some time, as a result that the measurements were to a great extent subjected to individual variation in spasticity. Yet plasmaconcentrations above 0.3 µg/ml dantrolene sodium gave consistently better results than placebo treatment in 6 out of 7 patients.

These positive results could not be confirmed, however, in a long-term open trial, including 25 patients (Chapter VII). In the latter study a beneficial effect could only be established in 7 patients. When we compared the daily dose of patients who did well on dantrolene sodium with the daily dose of the patients with no reaction or who became worse, there were significantly more patients with a positive result who remained on 100 mg daily than on a higher dose (up to 400 mg per day). Moreover, those high daily doses were not associated with significantly higher blood-levels of dantrolene sodium. Capacity limited absorption of dantrolene sodium from the intestine is the most likely explanation for these findings. These findings suggest that most patients who do not react favourably to 100 mg per day, will not react to a much higher dosage either.

Though dantrolene sodium caused improvement of passive resistance, reflex activity, and clonus in most patients, these effects were often not associated with improvement of the so called "overall clinical result". This discrepancy was also observed in some other trials (see Chapter III).

Since the "overall clinical result" is highly dependent on the experience and the expectations of both doctor and patient, it is useful to compare the effectiveness of dantrolene sodium with other known muscle relaxants in a double-blind fashion. This can circumvent these methodolocigal errors. In a double-blind cross-over trial Schmidt et al (1976) compared dantrolene sodium with diazepam, each administered for 2 weeks. 42 Patients with stable multiple sclerosis were treated with a maximum dose of either 400 mg dantrolene sodium or 20 mg diazepam. Both drugs reduced spasticity, clonus, and reflexes significantly and were considered to be equally effective. The dose preferred maximally did not exceed 225 mg per day. Levine and Van Brocklin (1977) compared, in a double-blind study, dantrolene sodium with baclofen and diazepam in 36 patients with spasticity involving two or more extremities. Baclofen was more effective than dantrolene sodium or diazepam in reducing spasticity associated with multiple sclerosis, but less effective in patients with spinal cord lesions.
As to the unwanted side effects of dantrolene sodium, the effect on the liver should be taken into account. In several case reports hepatic injury following dantrolene sodium therapy was reported (Ogburn et al, 1976; Schneider and Mitchell, 1976; Lundin et al, 1977; Goodman et al, 1977). Utili et al (1977) monitored 1044 patients on dantrolene sodium for at least 60 days and found an incidence of hepatic injury of 1.8%. Females were significantly more affected. All fatalities occurred in patients older than 30 years who had been using dantrolene sodium for at least 2 months; 85% had taken more than 300 mg per day. In our study we did not find any abnormalities of the liver function, probably because of the small number of patients participating in this study. We have found, however, many other side effects.

From the studies presented in this thesis we may conclude that dantrolene sodium also affects the rat heart in-vitro in concentrations that are also obtainable in-vivo. The pharmacokinetic behaviour of dantrolene sodium offers an explanation for its long-lasting effect.
In man plasmaconcentrations of dantrolene sodium are correlated with the decrease of twitch tension of the skeletal muscle. The drug is probably absorbed erratically, while the absorption capacity is limited. This led us to recommend maximal doses of 100-150 mg, since not much benefit may be

expected from higher daily doses.
Dantrolene sodium seems to be a helpful drug in the treatment of spasticity, but only in a relatively small proportion of patients; frequent side effects may further limit its use.

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Dit proefschrift zou niet tot stand gekomen zijn zonder de hulp van medewerkers van het Instituut voor Klinische Farmacologie, het Farmacologisch Laboratorium, de voormalige Werkgroep Spasticiteit, de Neurologische Kliniek van de Rijksuniversiteit te Groningen, Norwich Benelux B.V. te Utrecht, Eaton Norwich Laboratories te Norwich N.Y. , de revalidatiecentra "Beatrixoord" te Haren, "De Hoogstraat" te Leersum, "Heliomare" te Wijk aan Zee en het Medisch Fysisch Instituut TNO te Utrecht.
Ik dank allen hartelijk, die mij op enigerlei wijze bij de uitvoering en de presentatie van dit onderzoek hebben geholpen.